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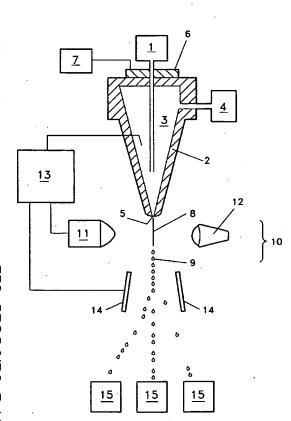
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(54) Title: INTEGRATED HERD MANAGEMENT SYSTEM UTILIZING ISOLATED POPULATIONS OF X-CHROMOSOME BEARING AND Y-CHROMOSOME BEARING SPERMATOZOA



(57) Abstract: Animal management system that utilizes artificial insemination (27) samples of isolated X-chromosome bearing and Y-chromosome bearing populations of spermatozoa to inseminate female animals (17) an embodiment of which can provide an alternative to traditional harvesting or marketing (20) of non-replacement heifers.



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INTEGRATED HERD MANAGEMENT SYSTEM UTILIZING ISOLATED POPULATIONS OF X-CHROMOSOME BEARING AND Y-CHROMOSOME BEARING SPERMATOZOA

5 I. TECHNICAL FIELD

Generally, herd management technologies utilizing isolated populations of X-chromosome bearing spermatozoa and Y-chromosome bearing spermatozoa that may be used with a variety of species of mammals. Specifically, an integrated bovine herd management system that utilizes isolated populations of X-chromosome bearing spermatozoa in a single-calf heifer system to increase the value of non-replacement heifers.

II. BACKGROUND

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the industry and researchers to evaluate various production systems. One conventional herd management system that has received considerable attention is the single-calf heifer system (SCH). This system has the capability to utilize non-replacement females normally targeted for slaughter. Simulated SCH systems compared to other beef management schemes using average costs of production and returns for products from 1958 to 1986 can be shown to be profitable. However, in order for a SCH system to remain economically sustainable, the end product must be acceptable to the consumer. The most essential component of the SCH system is that the heifer calve and be ready for harvest before she is 30 mo of age in order to avoid advanced carcass maturity.

Carcasses of advanced maturity pose problems in palatability, and therefore may be penalized by financial discounts. The USDA has set the approximate chronological age that corresponds to the physiological maturity score of "B" or greater to be 30 months of age or greater. However, maturity scores may increase with increasing chronological age at a much faster rate than USDA indicates and therefore suggested that animals 24 months of age and greater more accurately correspond to USDA maturity scores of "B" or greater. Therefore, to minimize risk of financial discounts and provide the consumer with a highly palatable product, target age of harvest for a SCH may be less than 24 months of

age.

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The SCH system can be designed to produce a carcass from the SCH as well as a calf. The carcass of the SCH must be of high quality but must not sacrifice the quality of the progeny. A production system in which a SCH is to rear a calf and be ready for harvest by 24 months of age, can possibly be accomplished by breeding the heifer at a non-traditional age of 9 months. Furthermore, it has been hypothesized that the younger the cow herd, the greater proportion of total feed used for weight production and a smaller amount of feed used for maintenance, lactation, gestation, and body condition score, hence increased biological efficiency.

It has also been hypothesized that if a SCH could produce only female calves, then only one parturition per female may be necessary and the dam could be slaughtered at a young enough age to still attain consumer acceptability without maturity discounts, thus creating a self-perpetuating herd system. Mating young cows to sexed-semen to yield female progeny may also reduce calving difficulties and increase calf survival.

To accomplish early breeding, induction of early puberty is necessary. Maturation into this state involves complex interactions between endocrine function, environment, social environment, breed, nutrition, and weight to bring about the development of reproductive tract and reproductive function. Diet is an effective tool to induce puberty. High-energy diets have been reported to induce onset of puberty in heifers at earlier ages than diets of lower energy. Weight of heifers tends to have a greater impact on puberty than age at puberty. Furthermore, heifers fed diets high in propionate production in the rumen reached puberty at lighter weights. Similarly, it has been found that diets containing ionophores decreased the age at onset of puberty, not related to ADG or body weight. Although plane of nutrition is inversely related to age at puberty, pattern of gain has no effect on age at puberty as long as heifers reach approximately 60-65% of mature body weight prior to breeding season. Hence, induction of early puberty depends on reaching target weights at a young age by feeding a feedlot-type diet high in energy and along with an ionophore. Early-weaning non-replacement heifers and managing them in feedlot conditions immediately following weaning is a means of reaching target weights

as early in life as possible.

Early weaning has proven to be a tool to increase dams BCS without detrimental effects on calves. It has been found that early-weaned heifers placed on high quality diets resulted in faster rates of gain than contemporaries that remained on their dams. Furthermore, early-weaned calves may be more efficient in feed conversion; in addition they had greater total gains from birth until slaughter.

Even though there have been a variety of innovations, as described in the foregoing, with respect to herd management technologies, conventional herd management systems including the SCH herd management system have significant problems that remain unresolved.

Averaged over thousands of animals, about half the number of births in a herd will be female and the half male. For example, in conventional beef cattle herds 49% of calves born will be heifers. Due to chance alone, however, it is not unusual to have only 40% heifers from 100 consecutive calvings.

As such, a significant problem with conventional herd management systems can be that at equilibrium about 40% of beef females must be breed for herd replacements to maintain herd size because over half the calves born are bulls, and some of the heifers born either die, become unthrifty, or do not become pregnant. With respect to the conventional SCH herd management systems, replacement females must be purchased to perpetuate the herd if females are harvested shortly after weaning of their calf.

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Another significant problem with conventional herd management systems can be that females average a lower weight and command a lower price at the time of sale than males under identical management. For example, under identical management, at weaning steers can average 519 pounds and garner an average of \$0.92 per pound, while heifers can average 491 pounds and garner an average of \$0.85 per pound. In this case, the steers provide a \$60.00 advantage at sale solely due to their sex.

With respect to the problems with conventional herd management systems and specifically with SCH herd management as above-described, the invention addresses each in a practical fashion.

5 III. DISCLOSURE OF THE INVENTION

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It is now possible to isolate populations of X-chromosome bearing or Y-chromosome bearing populations of spermatozoa. Certain technologies such as flow cytometry allow spermatozoa to be sorted with an accuracy of greater than 90%. Separated spermatozoa can be utilized to accomplish in vitro or in vivo artificial insemination of or fertilization of oocytes of numerous mammals such as bovids, equids, ovids, goats, swine, dogs, cats, camels, oxen, buffalo, or the like. See for example, WO 96/12171; WO 00/06193; WO 99/33956; and PCT/US01/15150, each hereby incorporated by reference.

Accordingly, a broad object of embodiments of this invention can be to provide herd management systems which utilize isolated populations of X-chromosome bearing or Y-chromosome bearing spermatozoa.

One aspect of this broad object of the invention can be to increase the percentage of female animals available to expand an existing herd or to sell as replacement heifers.

A herd management program utilizing populations of X-chromosome bearing spermatozoa of greater than 90% purity would allow a large surplus of female animals.

Another aspect of this broad object of the invention can be to increase selection intensity by allowing insemination of fewer but superior dams to produce replacement heifers. For example, in a beef herd at equilibrium about 40% of beef females must be breed for herd replacements to maintain herd size. With isolated populations of X-chromosome bearing spermatozoa, only 20% of females would need to be bred for replacements instead of the normal 40%, thus increasing selection intensity.

Another aspect of this broad object of the invention can be to breed females to bear females to decrease the incidence of birthing difficulty. A major problem on

ranches, for example, is dystocia when heifers calve. The majority of dystocias are due to bull calves that average about five pounds heavier than heifers. This can be minimized by using isolated X-chromosome bearing spermatozoa from sires that generate a low incidence of difficult births.

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Another aspect of this broad object of the invention can be to dispense with the conventional cow herd all together. Utilizing isolated populations of X-chromosome bearing spermatozoa, it can be possible to have every female replace herself with a heifer calf just before being fattened for harvest.

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Another aspect of this broad object of the invention can be to provide a terminal cross program that produces only males. In certain embodiments of this invention, cows could have substantially terminal cross bull calves by artificial insemination with isolated populations of Y-chromosome bearing spermatozoa of 90% or greater purity. In other embodiments of this invention, an all male terminal-cross system could be used by purchasing all replacement heifers.

Yet another aspect of this broad object of the invention can be to integrate earlyweaning, induced puberty, or sexed semen into a single-calf heifer system to increase value of non-replacement heifers. The integrated system would produce high quality products available to the consumer as well as provide a producer an alternative management system that has the potential to increase profitability.

Naturally further objects of the invention are disclosed throughout other areas of the specification and claims.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a generalized flow cytometer system used to sort X-chromosome bearing spermatozoa from Y-chromosome bearing.

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Figure 2 shows a second view of a generalized flow cytometer generalized flow cytometer system used to sort X-chromosome bearing spermatozoa from Y-chromosome bearing.

Figure 3 shows herd management system that uses traditional weaning methods.

Figure 4 shows a herd management system that uses early weaning methods.

Figure 5 shows an embodiment of the herd management invention using isolated Y-chromosome enriched populations of spermatozoa.

Figure 6 shows an embodiment of the herd management invention using isolated Y-chromosome enriched populations of spermatozoa and early weaned offspring.

Figure 7 shows an embodiment of the herd management invention using isolated X-chromosome enriched populations of spermatozoa.

Figure 8 shows an embodiment of an estrus synchronization protocol.

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V. MODE(S) FOR CARRYING OUT THE INVENTION

The invention involves herd management technology utilizing isolated X-chromosome bearing and Y-chromosome bearing populations of spermatozoa or sperm cells. X-chromosome bearing and Y-chromosome bearing populations of spermatozoa can comprise populations of intact live spermatozoa, or may also comprise frozen populations of X-chromosome bearing and Y-chromosome bearing spermatozoa. While particular examples of the invention are provided in the context of herds comprising beef cattle, it should be understood that the technologies described can have various applications with respect to a variety of species of mammal including, but not limited to, humans, bovids, equids, ovids, canids, felids, goats, or swine, as well as less commonly known animals such as elephants, zebra, camels, or kudu. This list of animals is intended to be exemplary of the great variety of animals from which spermatozoa can obtained and routinely isolated into X-chromosome and Y-chromosome bearing populations and to which this herd management invention can apply. As such, the examples provided are not intended to limit the description of the invention to the management of any particular specie(s) of mammal(s).

Now referring to Figures 1 and 2, an embodiment of the herd management system invention uses X-chromosome bearing and Y-chromosome bearing spermatozoa isolated by a flow cytometer. Flow cytometers used to isolate populations of X-chromosome bearing or Y-chromosome bearing spermatozoa can comprise a sperm cell source (1) which acts to establish or supply spermatozoa stained with at least one fluorochrome for

analysis. The stained spermatozoa are deposited within a nozzle (2) in a manner such that the stained spermatozoa are introduced into a fluid stream or sheath fluid (3). The sheath fluid (3) is usually supplied by some sheath fluid source (4) so that as the sperm cell source (1) supplies the stained spermatozoa into the sheath fluid (4) they are concurrently fed through the nozzle (2).

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In this manner it can be easily understood how the sheath fluid (3) forms a sheath fluid environment for the sperm cells. Since the various fluids are provided to the flow cytometer at some pressure, they flow out of nozzle (2) and exit at the nozzle orifice (5). By providing some type of oscillator (6) which may be very precisely controlled through an oscillator control (7), pressure waves may be established within the nozzle (2) and transmitted to the fluids exiting the nozzle (2) at nozzle orifice (5). Since the oscillator (6) acts upon the sheath fluid (3), the stream (8) exiting the nozzle orifice (5) eventually and regularly forms drops (9). Because the sperm cells are surrounded by the fluid stream or sheath fluid environment, the drops (9) may entrain within them individually isolated sperm cells.

Since the drops (9) can entrain sperm cells, the flow cytometer can be used to separate sperm cells based upon sperm cell characteristics. This is accomplished through a sperm cell sensing system (10). The sperm cell sensing system involves at least some type of detector or sensor (11) that responds to the sperm cells contained within fluid stream (8). The particle or cell sensing system (10) may cause an action depending upon the relative presence or relative absence of a characteristic, such as fluorochrome bound to the sperm cell or the DNA within the sperm cell that may be excited by an irradiation source such as a laser exciter (12) generating an irradiation beam to which the sperm cell can be responsive.

With respect to spermatozoa, the availability of binding sites for Hoechst 33342 stain is dependant upon the amount of DNA contained within each spermatozoon.

Because X-chromosome bearing spermatozoa contain more DNA than Y-chromosome bearing spermatozoa, the X-chromosome bearing spermatozoa can bind a greater amount of fluorochrome than Y-chromosome bearing spermatozoa. Thus, by measuring the

fluorescence emitted by the bound fluorochrome upon excitation, it is possible to differentiate between X-bearing spermatozoa and Y-bearing spermatozoa.

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In order to achieve separation and isolation of spermatozoa based upon the amount of light emitted, emitted light can be received by a sensor (11) and fed to some type of separation discrimination system or analyzer (13) coupled to a droplet charger which differentially charges each droplet (9) based upon the amount of DNA within the sperm cell within that droplet (9). In this manner the separation discrimination system or analyzer (13) acts to permit the electrostatic deflection plates (14) to deflect drops (9) based on whether or not they contain an X-chromosome bearing spermatozoa or a Y-chromosome bearing spermatozoa.

As a result, the flow cytometer acts to separate the differentiated spermatozoa (16) by causing them to be directed to one or more collection containers (15). For example, when the analyzer differentiates sperm cells based upon a sperm cell characteristic, the droplets entraining X-chromosome bearing spermatozoa can be charged positively and thus deflect in one direction, while the droplets entraining Y-chromosome bearing spermatozoa can be charged negatively and thus deflect the other way, and the wasted stream (that is droplets that do not entrain a particle or cell or entrain undesired or unsortable cells) can be left uncharged and thus is collected in an undeflected stream into a suction tube or the like as discussed in United States Patent Application 09/001,394, hereby incorporated by reference herein. Naturally, numerous deflection trajectories can be established and collected simultaneously.

Now referring to Figures 3 and 4, conventional herd management comprises a herd of dams (17) of varying age and calves (18) that that over thousands of calves can typically comprise about 50% females and about 50% males. As can be understood from the figures, typically a portion of the herd of dams (19) is sold to the marketplace (20) along with a portion of the heifers (21) and substantially all the steers (22). A portion of the heifers (23) can provide replacements for the dams sold to the marketplace (20) although some replacement animals can also be bought outside of the herd to normalize the herd or improve herd genetics. For convenience and economic efficiency, all the

dams can be brought into estrous at about the same time through a variety of estrous synchronization protocols (24). Figure 8 shows one type of estrous synchronization protocol and that protocol is more fully described in Example 1 below.

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In addition, as shown by Figure 4, the calves can be early weaned at about 95 days to about 125 days as compared to traditional weaning at about 200 to about 230 days. Early weaning can be good tool to increase the BCS of dams, provide faster weight gain in calves, and can provide more efficient feed conversion.

Now referring to Figure 5, a generalized herd management invention is disclosed which can be used with a variety of species of animals as above-described. The herd management invention utilizes isolated populations of Y-chromosome bearing spermatozoa (some portion of the X-chromosome bearing spermatozoa population has been removed to enrich the ratio of Y-chromosome bearing spermatozoa to Xchromosome bearing spermatozoa in the total population) to provide a terminal cross that generates a desired ratio of male offspring mammals (18) to female offspring mammals. As can be understood from Figure 5, in certain embodiments of the invention substantially all the offspring mammals can be male offspring Isolated populations of Ychromosome bearing spermatozoa from numerous species of mammals can be produces as described above. Isolated populations of Y-chromosome bearing spermatozoa can be differentiated based upon this sex differentiation characteristic and at least 70%, at least 80%, at least 90%, or even higher percentages, even at least 98% of a plurality of spermatozoa can have a sex determination characteristic corresponding to the same sex of offspring mammal. These isolated populations of Y-chromosome bearing spermatozoa can be used in the context of various estrous synchronization protocols (24) and artificial insemination protocols (27) including, but not limited to, those estrous synchronization protocols and artificial insemination protocols described in United States Patent Application No. 09/001,394 and 09/015,454, each hereby incorporated by reference, to inseminate the dams (17) and fertilize at least one egg within the female of a species of mammal. The sex of the offspring mammals produced (18) can be predetermined based upon the ratio of Y-chromosome bearing spermatozoa to X-chromosome bearing spermatozoa in the artificial insemination samples used to inseminate the female of the

species of mammal. Certain embodiments of the invention adjust the population of male offspring mammals to a percentage of male offspring mammals of at least 70%, at least 80%, at least 90%, or even greater. When isolated populations of X-chromosome bearing or Y-chromosome bearing spermatozoa are used in artificial insemination protocols (27), the number of non-frozen live spermatozoa can be selected such that the artificial insemination sample contains the desired number. In the context of inseminating bovine mammals, the number of isolated Y-chromosome bearing spermatozoa in the artificial insemination sample can be no more than 10 million, for example. A low number of spermatozoa from about 10% to about 50% relative to the typical number of spermatozoa in an artificial insemination sample may be used. In certain species of bovine mammals, such as beef cattle, the number of spermatozoa can be no more than 5 million, no more than 3 million, or can even be as low as no more than 500,000, no more than 250,000, and in some embodiments of the invention no more than between 100,000 to 150,000 spermatozoa. In certain embodiments of the invention, the spermatozoa can be frozen and subsequently thawed prior to use. The number of motile spermatozoa in a frozenthawed sample of spermatozoa may be reduced.

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Similarly, when the invention is used in the context of equine mammals, the artificial insemination sample can comprise live non-frozen spermatozoa having a number of no more than 25 million, no more than 15 million, no more than 10 million, or no more than 5 million. Similar numbers of spermatozoa may be frozen and subsequently thawed prior to use. Various protocols for the insemination of equine mammals are further disclosed by PCT/US99/17165, hereby incorporated by reference.

As described above, in the context of beef cattle, upon weaning male animals (22) sold to the marketplace (20) can generate more revenue than female animals under identical herd management. Where the sex of the offspring mammals produced is substantially male animals, replacement animals (25) may have to be obtained from an external source (26). In some embodiments of the invention, 20% of the dams (17) are replaced each year once the herd is normalized.

Now referring to Figure 6, the invention can further comprise early weaning of

male offspring (18) (or the desired sex ratio of offspring afforded by artificial insemination with populations of spermatozoa having known ratios of X-chromosome to Y-chromosome bearing spermatozoa). Understandable actual number of days to weaning of the offspring mammal can vary from species to species. Early weaning can be, with respect to beef cattle, as early as 95 days, or at an average age of about 110 days, or in certain embodiments of the invention between about 95 days to about 125 days. Additional embodiments of early weaned bovine mammal management are provided by Example 1 below.

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Now referring to Figure 7, a generalized herd management invention is disclosed which can be used with a variety of species of mammals. The herd management invention utilizes isolated populations of X-chromosome bearing spermatozoa (some portion of the Y-chromosome bearing spermatozoa population has been removed to enrich the ratio of X-chromosome bearing spermatozoa to X-chromosome bearing spermatozoa in the total spermatozoa population). By utilizing isolated populations of X-chromosome bearing spermatozoa (as many as 98 of 100 spermatozoa bearing an Xchromosome) to artificially inseminate females (17) in the herd, female offspring can be produced to replace substantially all (or the number desired) of the females (17) harvested from the herd (20). As such, in certain embodiments of the invention each female (17) can have a single parturition prior to being harvested (20). The herd management invention can further comprise the practice of induced early puberty. Early puberty can be induced by generating rapid weight gain in the mammal. As disclosed in further detail by Example 1, puberty can be induced in beef cattle as early as between about 250 days after birth to about 270 days after birth. A weight gain of about 1.3 kilograms per day to about 1.4 kilograms per day per head can be sufficient to induce early puberty. By inducing early puberty artificial insemination estrous synchronization (24) and artificial insemination (27) and can be performed at an earlier time in the herd management cycle. To further shorten the time between birth and harvest of the animal while allowing at least one parturition for replacement, the female mammal can be early weaned as described above. In a beef cattle embodiment of the invention a female can be born, weaned at between about 95 to about 125 days, estrous synchronized at between about 250 to about 280 days, artificially inseminated, calve about 9 months later and be

harvested prior to 24 months.

While Figure 7, provides a specific time line for beef cattle embodiment of the herd management invention, it is understood that is illustrative of the broad variety of species of mammal that can be managed in a similar fashion and the specific example and time line provided is not intended to limit the invention to that specific example of that time line.

Now referring to Figure 8, an exemplary estrous synchronization protocol for beef cattle is provided in which cattle feed is top dressed with MGA at 0.5 milligrams per female animal per day for 14 days. On day 33, each female animal is injected with PGF2a. Three days subsequent, each female is artificially inseminated.

EXAMPLE 1

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An integrated herd management system (IS) was designed to evaluate integration of early weaning and use of sexed semen in a single calf heifer (SCH) system to increase value of non-replacement heifers. The project consisted of five phases; Phases I, II, and III were developmental stages of the heifers. Phase IV was a qualitative measurement of the integrated system where carcass evaluation occurred. Phase V determined economic status of the integrated system. The integrated IS may be an alternative to the traditional marketing (TMS) of non-replacement heifers. Traditional marketing of non-replacement heifers is defined as the sale of TMS heifers on live-weight bases immediately following >traditional= weaning at age of approximately 7 months (200 days). Therefore, the IS is economically compared to the TMS. The IS incorporates reproductive factors such as puberty and breeding of heifers; therefore, replacement heifer counterparts meant for reproduction and managed in a traditional replacement system (TRS) are compared to the IS heifers for these factors only.

Colorado State University's Eastern Colorado Research Center at Akron, CO was
the site for research. The project began July of 1999 (YI) with a replication that began
August 2000 (YII). Data are presented for all phases of YI but stop after PI in YII as
research beyond that point had not been completed. Heifers from the Red Angus X

Hereford ECRC herd were divided into two treatment groups. Heifers in the IS were of non-replacement status, particularly those born in the last half of the calving season but no younger than 55 d of age on the date of early weaning. These heifers were early weaned, fed a high energy diet to promote rapid growth of approximately 1.6 kg/d in order to reach 65% of mature weight (determined to be 500 kg by data from herd dams) by 9 mo of age. The IS heifers were mated at approximately 10 mo of age and harvested by 24 mo of age (Table 1). All other heifers of replacement status were managed in the ECRC replacement heifer system, weaned at approximately 7 mo of age, developed on range, and mated at 14 mo of age.

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TABLE 1. Timeline of resear		phase and corre heifers.	esponding age	of Integrated
Phase I ¹ :Weaning-Breeding	Ye	ear I	Ye	ar II
Age in (d)	109 ± 15.0	0/7/28/1999	116 ± 10.5	08/07/2000
Age out (d)	322 ± 15.0	02/26/2000	316 ± 10.5	02/27/2001
Days on feed PI	213		200	•
Phase II ² :Breeding-Calving				
Age in (d)	320 ± 12.7	02/26/2000	316 ± 10.5	02/27/2001
Age out (d)	573 ± 12.7	11/8/2000	-	-
Total days	253		-	-
Phase III ² :Calving-Harvest				
Age in (d)	573 ± 12.7	11/8/2000	-	-
Age out (d)	719 ± 12.7	04/05/2001	•	
Total days	146			-
Data based on original Integrate Data based on final 22 hd of Integrate were fed to finish and harvested yet collected.	egrated System h	eifers. These he		•

Phase I: Weaning of Heifer to Breeding of Heifer Weaning

The first year's (YI) experimental group consisted of 46 IS heifers that were weaned at a non-traditional early age of 110 " 15.0 d and 40 TRS heifers were weaned at a traditional weaning age of 229 "2.8 d. The second year's (YII) experimental group consisted of 48 IS heifers weaned at an average age of 115 " 26.9 d and 48 TRS heifers traditionally weaned and weighed at 174 " 21.2 days of age. Body weight of heifers was

recorded at each weaning date. Body condition scores (9-point scale) of the dams were recorded at the time of early weaning in YI and YII then again at the time of traditional weaning.

5 Nutritional Management

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Dams of the heifers were managed on native range in two separate pastures in YI. Dams of the TRS heifers were allowed winter supplementation whereas dams of the IS heifers were not. The dams were managed in this manner to allow evaluation of an earlyweaning program on winter feed expenditures. Weaning strategy had an affect dam BCS at time of TW, where the dams of the EW calves had greater BCS than the dams of the TW calves in YI only. The dams of the IS heifers were managed without supplementation as body reserves were adequate enough to allow the dams to lose body condition without risk of health or production loss. The dams of the TRS heifers were managed to allow body weight gain or maintenance. The dams were combined at the end of the winter season at similar body weights and condition. However, in YII, the dams were not managed separately as in YI because BCS of dams were equal at time of TW. Drought conditions limited forage quality and availability to dams; hence, weaning of the TRS occurred 55 d earlier than TW of TRS in YI. The combined effect of drought conditions and shorter duration of lactation of the dams of the TRS heifers in YII may explain lack of BCS difference between dams of IS heifers and dams of TRS heifers. Equal body weights did not allow any contrast between weaning strategies according to winter supplementation and therefore eliminated the need to manage dams separately. Pasture lease and feed cost for dams were analyzed.

Traditional replacement system heifers were managed on triticale pasture with access to native range in YI. In YII, TRS heifers were managed in dry lot conditions due to insufficient grass on native range resulting from drought conditions. Dry lot ration was balanced according to NRC (68) and included whole shelled corn, millet hay, and alfalfa hay. Nutritional values for triticale (63) and native range (19) and are listed in Tables 2,3, and 4. Nutritional values are based on 100% inclusion in the diet as amount of each forage in the diet can not be established, however, triticale was likely the main source of nutrition. Ration samples of the IS heifer diets were periodically collected throughout

Phase I and III and analyzed by Olsen's Laboratory McCook, NE (Figures 1,2,3 and 4).

	Pr	airie Sand	Reed	S	and Blueste	m		Blue Gram	ıa		Sun Sedg	e
	% Diet	Crude	Crude	% Diet	Crude	Crude	% Diet	Crude	Crude Protein	% Diet	Crude	Crude
	(DM)	Fiber %	Protein %	(DM)	Fiber %	Protein %	(DM)	Fiber %	%	(DM)	Fiber %	Protein %
Oct-Nov	1.1	38.2	2.8	0.5	32.4	3.7	31	25.5	5	1	30.5	6.1
Dec	1	36.8	2.5	0	33	3.5	71	29.3	4	1	32.15	5.6
Jan ·	3.5	37	2.6	0.5	34.9	3.7	73.5	29.95	3.7	2	31.9	5.5
Feb	2.5	35.7	2.8	0.5	35	2.8	62.5	31.15	4.2	1	27.4	6.8
March	4.5	39.15	2.5	0	38.5	3.8	37.5	30.9	5	1.5	24.35	5.7
April	4	38	3	0	35.5	4.3	13	27.15	5	1	28.3	9.3
May	0.3	33.65	16.6	0	35.6	4.5	0.4	29.1	7	1.5	24.1	14
June	68	35.3	13.6	3.8	30.9	12.8	0.5	28.7	10.2	9.1	22.45	12.6
Late June	64	38.27	11	14	36.48	10.9	2	30.32	8.5	0	27.29	10.5
July	75	35.69	7.7	12	33.16	9	7	30.32	7.9	1	27.71	9.6
Aug	66	36.73	7.2	9	29.37	7.9	14	30.63	7.5	0	27.43	8.4
Sept	32	35.41	6.3	22	31.46	6.4	35	30.71	5	1	28.03	6.7
Late Sept	34	35.13	6.1	0	30.76	5.9	43	30.91	4.3	0	28.42	5.7
Oct	1	31.02	3.4	0	33.23	3.6	39	32.47	3.1	2	29.95	5.4 •

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	Need	lle-and-thre	ad	Wes	tern Wheat	grass		Forbs			Other	
	% Diet (DM)	Crude Fiber %	Crude Protein %	% Diet (DM)	Crude Fiber %	Crude Protein %	% Diet (DM)	Crude Fiber %	Crude Protein %	% Diet (DM)		Crude Protein %
Oct-Nov	56.5	35.3	4.8	2	35.6	3.2	6	25.2	6.5	2	-	-
Dec	21	34.8	4.3	4	33.45	2.2	1	28.3	5.4	1	-	-
Jan	16.5	36.85	3.4	0	33.65	2.7	1	26.85	5.1	3	-	-
Feb	26.5	33.15	3.7	2	27.65	3.2	1	27	5.6	4	-	-
March	35.5	34.55	3.8	18.5	33.1	4.4	0	29.2	4.7	2.5	-	-
April	61	29.8	8.2	21	33.3	7.1	0	31.6	8	0	-	-
May	33	28.7	12.3	63.2	24.9	17.3	0.4	22.6	18.8	1.2	-	-
June	10.9	31.85	10.5	0.5	35	12.5	2.6	20.1	19.8	4.6	-	-
Late June	4	36.01	8.3	10	34.3	14.1	4	25.36	12.9	· 2	-	-
July	2	33	8.8	1	31.85	12.8	1	25.1	14	1	-	-
Aug	2	32.3	9.5	0	29.7	11.4	1	23.39	11.6	8	-	-
Sept	1	34.04	6.3	0	29.6	8.1	3	20.95	10.2	6	-	-
Late Sept	6.5	33.83	6.2	7	25.2	9.9	6.5	18.44	10.2	3	-	-
Oct	45	33.6	5.3	9	29.3	6	3	23.7	6.6	1	_	

TABLE 3. Seasonal trends in crude protein and TDN content of triticale forage as evaluated by clippings^a.

Crude Protein TDN (%) (%)

February 21.1 74.2

June 19.4 75.3

aMount (2000).

FIGURE 1. Variation in dietary digestible protein, crude protein and TDN Year I:Phase I

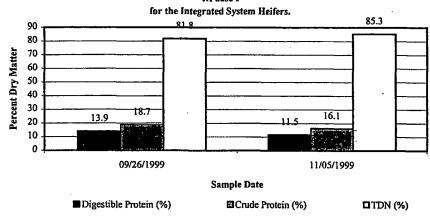
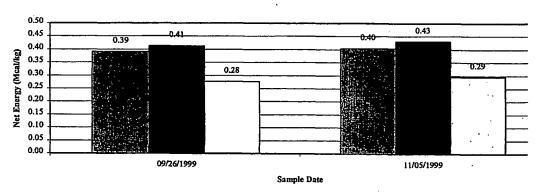
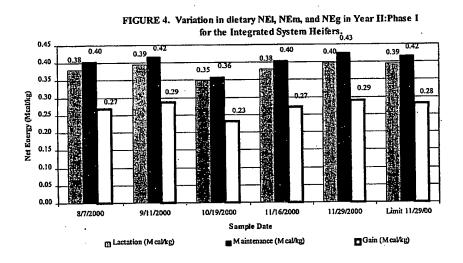


FIGURE 2. Variation in dietary NEI, NEm, and NEg Year I:Phase I for the Integrated System Heifers.



☐ Lactation (Mcal/kg) Maintenance (Mcal/kg) ☐ Gain (Mcal/kg)

FIGURE 3. Variation in dietary digestible protein and TDN in Year 1: Phase III and Year II: Phase I for the Integrated System Heifers. 100.0 73.7 Percent Dry Matter 80.0 60.0 40.0 12.16. 11.15. 20,0 11/29/2000 10/19/2000 Limit 11/16/2000 08/07/2000 11/29/00 Sample Date **TDN** (%) ☐ Crude Protein (%) Digestible Protein (%)



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The IS heifers were managed in feedlot immediately following weaning and continuing for 213 d and 200 d in YI and YII, respectively. Self-feeders were utilized for 140 days in YI and 5 d in YII then bunk-fed for the remainder of Phase I (73 d YI and 195 d YII). The duration of self-feeders utilized in YII was limited due to sickness and necessity to administer medicated feed. The ingredients of the feedlot ration YI included triticale grain, sunflower meal pellet, corn ground alfalfa, protein supplement and Rumensin7. Ingredients of the feedlot ration in YII were similar to YI with the exclusion of sunflower meal pellet. Weight of the EW heifers was measured every 28 d and the ration evaluated and adjusted according to heifer gain. The most important goal of the feeding strategy was for IS heifers to reach 65% of mature weight (based on herd of

origin mature weight of 500 kg) by 9 mo of age to induce an early puberty. Therefore each 28 d interval had a goal of 1.36 kg/day gain until heifers began to cycle. At this point the ration energy density was reduced to prevent over fattening and possible subsequent reproduction/calving difficulties. Daily individual intake was calculated by dividing pen intake by total animals in the pen (Figure 5 and 6). Rations were balanced according to NRC (68) requirements for growing/finishing calves at 1.3 kg/d gain.

FIGURE 5. Year I daily dry matter intake (kg/hd/d) of Integrated System heifers in Phase I.

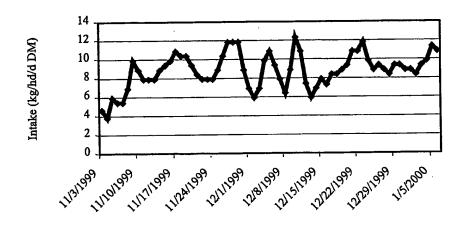
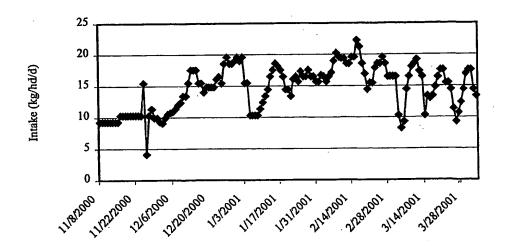


FIGURE 6. Daily intake of Integrated System Heifers Year I:Phase III.



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Heifers that had poor gains and/or exhibited chronic morbidity were culled from the system. Culled heifers were sold at a local livestock auction at market price.

Mortality of heifers was accounted for in the economic analysis. Weight of dead animals was estimated according to the group average. A dollar value of the dead animals was calculated by market purchase price of an animal of similar weight and age.

Monitoring Onset of Puberty

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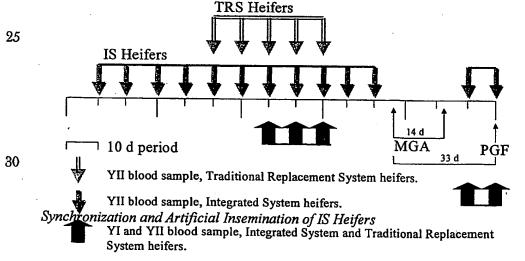
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Onset of puberty and estrous was monitored by behavioral and physiological indicators. The DDx Electronic Heat Watch7 system with the aid of 3 (YI) and 1 (YII) androgenized cows monitored behavioral patterns and the onset of standing heat via mount duration and frequency (96). Androgenization was accomplished by methods described by Nix et al. (67). Androgenization of cows was conducted due to the hypothesis that androgenized cows have a similar effect on enhancing puberty through pheromonal cues as hypothesized for bulls. Jugular blood samples of IS heifers were taken at 10 d intervals for a period of 2 mo (YI) and 3 mo (YII) prior to MGA/PGF synchronization and again 10 d prior to and on day of PGF injection (Figure 7). Serum samples were analyzed for progesterone by radioimmunoassay (21). Percent of TRS heifers at puberty was also measured by progesterone assay for one month prior to MGA/PGF synchronization of the IS heifers. Heifers were considered pubertal when serum progesterone concentration was greater than 1 ng/ml within a 10 d period (7).

FIGURE 7. Blood sampling protocol for Year I and Year II.



The IS heifers underwent estrous synchronization accomplished by top dressing feed with 0.5 mg MGA per hd/d for 14 d followed by PGF injection 19 d after the last day of MGA feeding as described by Deutscher (20). Heifers were synchronized at 250 " 15.0 d of age YI and 250 " 14.9 d of age YII. Heifers were AI by one of two technicians following standing estrus up to 72 h post PGF injection according to a.m./p.m. protocol. At 72 hr post PGF injection, all remaining pubertal heifers were mated at a fixed-time. A breeding period of 45 d (YI) allowed heifers three or four opportunities to be AI and 24 d (YII) allowed 2 matings. All rebreeds were based on standing heat recorded by Electronic Heat Watch7 and/or visual observation and bred according to a.m./p.m. protocol.

Semen used for artificial insemination was collected from two Black Angus bulls (YI) and one Black Angus bull (YII) with low birth weight EPD of 0.5, 1.5 and B 1.43, YI and YII, respectively. Semen was sorted using flow cytometry, selected for X-chromosomal sperm (82). Semen doses for insemination contained three million sperm (YI) and six million sperm (YII) per dose with at least 35% post thaw motility.

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During YI, heifers were fixed-time mated with sexed semen, randomly AI with one of two sires as determined by random order of the heifers entering the breeding box; sires were alternated with every heifer. Heifers that required a second mating were inseminated with sexed semen from the same sire used in the fixed-time mating. Heifers that required a third mating were randomly inseminated with either sexed or non-sexed semen by one of two sires. Non-sexed semen was used to mate 6 IS heifers and was a breech of protocol. Seven IS heifers exhibited a fourth standing heat during the 45 d breeding season, 1 heifer was mated to sexed semen and the remaining 6 heifers were mated by natural service of a bull. Likewise, natural service with a bull was a breech of protocol.

The YII IS heifers were fixed-time mated to one sire to minimize variation of progeny. Protocol for the second breeding season called for one-third of the YII IS heifers to be inseminated with non-sexed semen and the remaining 2/3 inseminated with sexed semen to compare fertility of sexed versus non-sexed semen. Mating of the heifers

to non-sexed versus sexed semen was determined by random order of the heifers entering the breeding box; every third heifer was bred to non-sexed semen. Likewise, 1/3 of the heifers that required subsequent breeding called for insemination with non-sexed semen; the remaining heifers were inseminated with sexed semen. The final result is that fixed-time insemination of IS heifers was done with 25.4% of heifers mated to non-sexed semen; the remaining 74.6% of heifers were mated to sexed semen. After 24 d of the breeding season, 28.6% of heifers were inseminated with non-sexed semen and the remaining 71.4% were inseminated with sexed semen.

10 Diagnosis of Pregnancy

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First service conception rate was determined by ultrasonography 34 d and 45 d post fixed-time mating in YI and YII respectively. Overall conception and pregnancy rates were also determined by ultasonography 34 d and 60 d following the last date of insemination for YI and YII respectively. Heifers diagnosed non-pregnant were culled from the system and the remaining pregnant heifers went on to Phase II. Culled heifers in YI were sold at market price to the ECRC feedlot. Revenue created by this sale was accounted for in PI and used in the final economic analysis.

Phase II: Breeding to Calving (YI IS heifers)

20 Nutritional Management

The IS heifers were turned on to native range at an average age (calculated from the final 22 IS heifers) of 297 " 12.6 days of age. The heifers remained on pasture for 237 days at which time the first IS heifer gave birth (534 " 12.6 d of age). Weight of IS heifers were recorded on the first and last day of this phase. Forage nutritional values are reported in Tables 2 and 4 (19).

TAE	LE 4.	Seasor	al tren	ds in c	rude fil	ber and	crude pr	otein of	native r	ange clip	pings as	a result (of varia	ble se	asonal	intake	·*.
		Yea	r I, Pha	se i							Ye	ar I, Phas	e II				
												Year II,	Phase	П			
	Late		Oct-								Late				Late		Oct
		Oct	Nov	Dec	Jan	Feb	Mar	Арг	May	June	June	July			Sept		No

(DM)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Crude																			
Fiber %	30.25	30.40	32.04	30.96	30.42	30.47	30.42	32.10	30.50	25.88	31.53	36.09	34.38	32.05	30.25	30.40	32.04	30.96	30.42
Crude																			
Protein %	5.61	5.68	4.47	4.83	3.97	3.55	3.88	4.24	7.36	15.36	12.65	10.99	7.95	6.82	5.61	5.68	4.47	4.83	3.97
Adapted fro	m Denha	ım (19	65).																

Back-fat' Measurements

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Back-fat thickness was measured by ultrasound for the IS heifers 20 d prior to first date of calving and again 20 d following calving. Back-fat was measured between the 12th and 13th rib at: the distance of the ribeye with an Aloka 500V ultrasound machine.

Calving Management

The IS heifers calved in dry lot conditions. Heifers were observed every 4 hours during the calving season. Heifers received assistance from the calving manager if calf presentation or parturition progression was abnormal or unsatisfactory. Calving ease, calf vigor, calf birth-weight, sex, mortality and morbidity were documented. Calving ease and calf vigor scores are reported as follows: calving ease: 1= no assistance, 2= assisted, easy, 3= assisted, very difficult, 4=caesarean delivery, 5= breech birth, abnormal presentation: calf vigor 1= nursed immediately, calf was healthy and strong, 2= nursed on its own, but took some time, 3=required some assistance to suckle, 4=died shortly after birth, 5=dead on arrival.

In YI abortion occurred in 4 of the 25 IS heifers bred (16%). These IS heifers experienced early fetal loss and three were rebred by natural service as a result of IS heifers commingling with the normal herd during breeding season. These IS heifers were culled from the IS and sold as bred heifers at the local livestock auction in Yuma, CO. Revenue (\$650.00 per IS heifer) from these late-bred IS heifers was received by the IS and is included in the economic analysis. The forth IS heifer that experienced fetal loss did not rebreed and was kept in the IS, finished and harvested with her pregnant IS heifer counterparts.

Phase III: Calving to Slaughter

Nutritional Management

The IS heifers were placed on feedlot ration at 534 " 12.6 d of age until 696 " 12.6 d of age for 162 d. Ingredients included triticale grain, whole shell corn, and alfalfa (Figure 3 and 4). The ration was balanced according to NRC (68) requirements for lactating 550 kg cows and adjusted according to IS heifer and calf performance. Daily intake was calculated by dividing group intake by the number of animals in the pen (Figure 8). Integrated system heifer and IS calf weight was recorded every 28 d.

10 Weaning

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Calves born to the IS heifers were weaned at 120 " 19.6 d of age and marketed at a local livestock auction immediately following weaning. Revenue from the calves was accounted for in the final economic analysis.

Determination of Harvest

IS heifers remained in feedlot for 141 d and harvested at 696 " 12.6 d of age d (23 mo) of age. Integrated System heifers were considered market ready according to estimated visual backfat depth of 1.27 cm. The time from weaning to harvest was 21 d to allow adequate time for udder to cease lactation and undergo involution.

Phase IV: Carcass Evaluation

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Marketing of IS heifers

The final 22 IS heifers were marketed on formulated price according to carcass quality (live-weight, USDA Quality Grade and USDA Yield Grade). Premiums and discounts associated with the grid are listed in Table 5.

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	_	Prime	Choice	Select	Standard	Commercial	Utility	Canner	Dark Cutter	B-Maturity
		6.97	\$0.97	(1.87)	(\$11.87)	(\$25.00)	(\$75.01)	(\$75.01)	(\$22.84)	(\$11.87)
BASE	\$127.01									
1	\$3.00	136.98	130.98	128.14	118.14	105.01	55.00	55.00	107.17	118.14
2	\$1.50	135.48	129.48	126.64	116.64	103.51	53.50	53.50	105.67	116.64
3	(\$1,00)	132.98	126.98	124.14	114.14	101.01	51.00	51.00	103.17	114.14
4	(\$20,00)	113.98	107.98	105.14	95.14	82.01	32.00	32.00	84.17	95.14

5	2f. o	108.98	102.98	100.14	90.14	77.01	27.00	27.00	79.17	90.14
*Formula	ted pricing	per hundred	l lb.							

	•					Commerc			Dark	
		Prime	Choice	Select	Standard	ial	Utility	Canner	Cutter	B-Maturity
		\$15.37	\$2.14	(S4.12)	(\$26.17)	(\$55.12)	(2)023	(5165.3 71	·S50.25)	(S26.17)
BASE	\$280.01									
1	\$6.61	301.99	288.76	282.50	260.45	231.51	121.25	121.25	236.27	260.45
2	\$3.31	298.68	285.45	279.19	257.15	228.20	117.95	117.95	232.96	257.15
3	·\$2.20)	293.17	279.94	273.68	251.64	222.69	112.44	112.44	227.45	251.64
4	(\$44.09)	251.28	238.06	231.79	209.75	180.80	70.55	70.55	185.56	209.75
5	(\$55.12)	240.26	227.03	220.77	198.72	169.78	59.52	59.52	174.54	198.72

Carcass Data Collected

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Carcasses were tracked from the kill floor to the cooler on the day of harvest.

5 Approximately 36 h postmortem, USDA Quality Grade factors (skeletal maturity, lean maturity and marbling) and USDA Yield Grades (longissimus muscle area, hot carcass weight, and estimated percent of kidney pelvic and heart fat) were recorded (103). Strip loins were collected from each IS heifer carcass. Loins were taken to Colorado State University, aged for 14 days at 2EC then frozen (-29E C) until strip loin sections were sawed into steaks (2.54 cm thick).

Trained Sensory Evaluation and Warner-Bratzler Shear Force Values

Strip loins were removed from the freezer cut into 2.54 cm steaks which were then thawed in a refrigerated cooler (4EC) for 24 hrs. Steak temperature was monitored to ensure steak temperatures were between 1EC and 5EC immediately prior to cooking. Steaks were cooked to 70EC internal temperature using a Magikitch=n belt grill (Magigrill model TBG-60; Magikitch=n Inc., Quakertown, PA); (top heat=177EC, bottom heat=177EC, preheat=disconnected, height=1.85cm, cook time=6.55 min). Final endpoint temperatures were monitored using a handheld thermometer (Omega model HH21 thermometer; Omega Engineering, Inc., Stamford, CT). Cubed samples of each cooked steak were served to a sensory panel. Panelists were trained for two weeks according to procedures outlined by Meilgaard et al. (57) and AMSA (3). Panelist scored

the samples for juiciness, muscle fiber tenderness, overall tenderness, connective tissue and amount of flavor intensity using an 8-point scale (3).

Steaks for the Warner-Bratzler shear force values were handled and prepared in the same manner as stated above. The steaks were allowed to cool to room temperature (24EC) before removing six to ten cores (1.27 cm in diameter) parallel to the steak muscle fiber (3). Each core was sheared once using Warner-Bratzler shear machine. Individual peak shear force values were averaged to obtain a final representative shear force value for each steak. The WBS threshold of 4.5 kg determined carcass classification of tough or tender (89). Carcasses with WBS values greater than 4.5 kg were considered "tough", whereas carcasses with WBS values less than 4.5 kg were considered "tender".

Phase V: Economic Analysis

Total income and expenditures were recorded for each phase. Gross revenue/loss and net revenue/loss were calculated for each phase as well as final gross revenue/loss and net revenue/loss for all phase (Tables 14-19). Revenue/loss was reported for the entire system as well as revenue/loss per heifer. Economic analysis compared a traditional management system of non-replacement females to the IS. An additional simulation were conducted to compare and contrast effect of increased pregnancy rate (58%, 60%, 70%, 80%, 90% and 100%) and calf survival (88%) on profitability of the IS.

Phase I

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In PI, IS heifers were purchased into the system according to the seasonal live-weight markets in the area (\$103.00/cwt) (Table 14). Cull cows were purchased at market price, and androgenized to aid in heat detection. These cull cows were later sold on live-weight basis; prices reported are actual prices received. Integrated System heifers were realized from the system due to poor performance and sold at seasonal live-weight market price for the area. Economic loss due to death was considered equal to purchase price multiplied by weight of IS heifer at death. Dead IS heifers were assigned a weight based on the group average weight at the time of death.

TABLE 14. Income s	tatement for Year I Phas	se II.		
REVENUES	# Head Weight (cwt	:) ·	7	Cotal
Cull Open IS Heifer	18	. 10	\$66.00	\$11,880.0
GROSS INCOME EXPENSES				\$11,880.0
Pasture	# Head Lease/AUM	. 1	Months 7	Fotal
	43	\$13.00	1.4	\$508.6
IS Heifer (.65 AUM)	25	\$13.00	6.4	\$1,352.0
TOTAL				\$1,860.6
TOTAL OPERATING EXPENSE				\$1,860.6
NET REVENUE/LOSS (Income-expenses)				\$10,019.3

Feed cost was calculated by multiplying the cost of feed per ton by the total amount of feed consumed. Cost of feed per ton was marked up 10%. Yardage was charged at a rate of \$0.20 per head per day. Health costs included initial processing; hospital drugs administered to sick animals and associated chute charges of \$1.00/hd. Breeding costs were calculated by adding the total cost of synchronization drugs to the cost of semen, semen sorting fees, and technician wages. Gross revenue/loss for PI was calculated by subtracting the total expenditures from the total income and value of IS heifers remaining in the system.

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Breeding costs were calculated by summing cost of insemination and synchronization. Cost of insemination was calculated by multiplying the total number of inseminations by technician fee (\$4.00/insemination), sorting fee for semen (\$20.00/straw), and semen (\$12.00/straw). Synchronization drugs included MGA (\$1.96/hd) and prostaglandin (\$2.10/hd) for 43 head of IS heifers.

In YI, cost of maintenance for dams of TMS heifers and for IS heifers was calculated by adding the pasture lease cost to supplemental feed cost. The difference between the two groups winter feed cost was calculated.

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Phase II

Phase II gross and net revenue/loss was calculated by multiplying pasture lease cost by .65 AUM per month at a rate of \$13.00/AUM (Table 15). All heifers were moved

into PII for 1.4 mo, however only the 25 pregnant IS heifers remained in the PII for the duration of 253 d. Revenue generated from sale of open IS heifers are accounted for in this phase.

TABLE 15. Income s	statement for Year	I Phase	ш.	
REVENUES				
	# Head Ave V	VT	Market Price	Total
Cull Bred IS Heifers	3		\$650.00	\$1,950.00
Lightweight IS calf	1	1.2	100	\$120.00
Heavyweight IS calf	13	3.3	\$122.00	\$5,233.80
Finished IS Heifer	22			\$21,510.53
TOTAL				\$28,814.33
GROSS INCOME			•	\$28,814.33
EXPENSES				
Livestock	#Head Ave \	WT .	Market Price	Total
Death Loss of IS Calf	6	3.3	\$122.00	\$2,415.60
TOTAL				\$2,415.60
Feed	# Head Intake	e (ton)	Price/ton	Total
Com	22	41.101		
Millet Hay		1.533		-
Alfalfa Hay		8.52		
Medicated Feed		. 0	\$120.00	
Supplement 517		4.227	\$205.00	
Markup		55.382	\$15.00	
TOTAL			,	\$6,206.31
Yardage	#Head Rate		# Head Days	Total
Head In	22	\$0.30	3212	\$963.60
Dead	0	\$0.30	0	\$0.0
Realized	0	\$0.30	0	\$0.0
TOTAL	22		0	\$963.60
Health	# Head Rate			Total
Processing	46	\$1.00		\$46.00
Hospital drugs				\$0.0
Chute Charge				\$0.0
TOTAL				\$46.00
Marketing	# Head Rate			Total
Transportation	22	12.95		\$284.90
Brand Inspection	22	1.45		\$31.90
TOTAL	•	_		\$316.80
TOTAL OPERATING EXPENSE				\$9,948.31
NET REVENUE/LOSS (Income-expenses)				\$18,866.02
,,	•			+,

Phase III

Phase III economic analysis resembled PI for health costs and feed cost. Yardage was increased to \$0.30 per head per day as a result of increased labor associated with calves of IS heifers (Table 16). Cull cattle included IS heifers that were bred late and marketed as bred heifers. Prices reported are actual prices received from sale. Calves of the IS heifers were sold immediately following weaning on a live-weight basis. Prices reported are actual prices received. Prices reported for the remaining IS heifers were received upon marketing of heifers according to carcass merit (Tables 5 and 17). Gross revenue/loss was calculated by subtraction of total expenditures from total income.

Overall gross and net revenue/loss was calculated by summing gross and net revenue/loss

Overall gross and net revenue/loss was calculated by summing gross and net revenue/loss from each phase respectively.

QG and YG	Price Per 100 kg	Head	Wt (100 kg)	Total	QG and YG	Price Per 100 lb	Head	Wt (100 lb)	Total
Choice YG 2	285.45	6	21.17	\$6,042.83	Choice YG 2	129.48	6	46.67	\$6,042.83
Choice YG 3	279.94	8	29.58	\$8,281.64	Choice YG 3	126.98	8	65.22	\$8,281.64
Choice YG 4	238.06	1	3.79	\$902.23	Choice YG 4	107.98	1	8.36	\$902.23
Select YG 1	282.50	1	3.41	\$962.33	Select YG 1	128.14	1	7.51	\$962.33
Select YG 2	279.19	2	6.16	\$1,719.77	Select YG 2	126.64	2	13.58	\$1,719.77
B-Mat YG2	257.15	2	6.87	\$1,767.10	B-Mat YG2	116.64	2	15.15	\$1,767.10
B-Mat YG3	251.64	1	3.85	\$967.91	B-Mat YG3	114.14	1	8.48	\$967.91
B-Mat YG4	209.75	1	4.13	\$866.73	B-Mat YG4	95.14	1	9.11	\$866.73
TOTAL				\$21,510.53	TOTAL				\$21,510.53

TABLE 17. Comparison of net revenue of the Integrated S non-replacemen	
Integrated Heifer System	
Gross Revenue	\$43,285.81
Expense	\$43,200.97
NET REVENUE/LOSS (Income-expenses)	\$84.84
Traditional Management System	
Gross Revenue ¹	\$21,834.80
Expense ²	\$19,352.58
NET REVENUE/LOSS (Income-expenses)	\$2,482.22
Difference between Systems (IS-TMS)	(\$3,944.67)
Gross Revenue calculated by multiplying average weaning market price of \$88.00/cwt.	g weight of traditional weaned calves in YI by
Expense is equal to the five-year average of cow cost of the	ne ECRC herd.
Prices based on 43 head of heifers.	•
	

Difference in Profitability Between TMS and IS

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Profitability of the IS over the TMS was calculated by subtracting the net revenue of the TMS from net revenue of the IS (Table 18). Gross revenue/loss for TMS was calculated by multiplying the average weaning weight of the TMS heifers at the time of traditional weaning by a seasonal live-weight market price for the area (\$88.00/cwt).

regnancy Rate	Total Pregnant		Total	Revenue/Heifer	Revenue/Pregnai
58%	25	PI	\$(28,800.49)	\$ (626.10)	\$ (1,152.02)
		PII	\$10,062.15	\$218.74	\$402.49
		PIII	\$26,447.21	\$574.94	\$1,057.89
		Total	\$7,708.87	\$167.58	\$308.35
		Revenue above TMS	\$3,679.36	\$85.57	\$147.17
60%	26	PI	\$ (28,800.49)	\$(626.10)	\$(1,116.30)
		PII	\$9,448.05	\$205.39	\$366.20
		PIII	\$27,392.41	\$595.49	\$1,061.72
		Total Revenue above	\$8,039.96	\$174.78	\$311.63
		TMS	\$4,010.46	\$93.27	\$155.44
70%	30	PI	\$(28,800.49)	\$(626.10)	\$ (956.83)
		PII	\$6,377.50	\$138.64	\$211.88
		РШ	\$32,118.41	\$698.23	\$1,067.06
		Total	\$9,695.42	\$210.77	\$322.11
		Revenue above			
		TMS	\$5,665.91	\$131.77	\$188.24
80%	- 34	PI	\$ (28,800.49)	\$ (626.10)	\$(837.22)
		PII	\$3,306.96	\$71.89	\$96.13
		PIII _	\$36,844.41	\$800.97	\$1,071.06
		Total	\$11,350.88	\$246.76	\$329.97
		Revenue above TMS	\$7,321.37	\$170.26	\$212.83
90%	39	PI ·	\$(28,800.49)	\$ (626.10)	\$ (744.20)
		PII	\$236.41	\$5.14	\$6.11
		PIII	\$41,570.41	\$903.70	\$1,074.17
•		Total Revenue above	\$13,006.33	\$282.75	\$336.08
		TMS	\$8,976.83	\$ 208.76	\$231.96

100%	43	PI	\$ (28,800.49)	\$ (626.10)	\$ (669.78)
		PII	\$ (2,834.13)	\$ (61.61)	\$ (65.91)
	•	PIII	\$46,296.41	\$1,006.44	\$1,076.66
		Total	\$14,661.79	\$318.73	\$340.97
		Revenue above TMS	\$10,632.28	\$247.26	\$247.26
			010,052.20		0217.20

^{*}Assumes the Integrated System heifer death and realizer rate same as in experimental Year I and calf death rate of 2%.

Simulations

Simulations were conducted to evaluate the effect of pregnancy rate and increased calf survival would have on profitability (Table 19). Simulations varied pregnancy rates at 58% 60%, 70%, 80%, 90% and 100% to evaluate its affect on profitability of each phase and as a system. The pregnancy rate simulations assumed sexed-semen was utilized, 2% calf death loss and IS heifer death loss and realizer rate to be the same as in YI. Further assumptions included all IS calves to sell as >heavy-weight= (330 lbs) and no late-bred IS heifers occurred. Revenue from finished IS heifers based on the percent of each USDA quality and yield grade carcasses received in YI. Feed consumed calculated by multiplying the number of animals in pen average individual intake, based on YI results. All simulations are based on YI actual expenses and incomes.

15 Statistical Analyse

Statistical analyses were completed using the general linear model (GLM) procedure of SAS (81) and when appropriate, means were separated using Tukey's HSD (81) to determine differences in weights, age, and BCS across years. Logistic regression and contrasts (81) were used with first service or second service resulting pregnancy as the dependent variable; group and heat cycles or group and technician were considered independent variables respectively, to compare and contrast technician, sire, and semen effects within and across years. Group refers to the combination of sexed or non-sexed semen from one of three sires to yield four groups, one sire (YI) with both sexed and non-sexed semen used for insemination of IS heifers, and two sires (YII) with only sexed semen used for insemination of IS heifers. Chi square and correlation analyses (81) were conducted on taste panel characteristics (maturity, session, juiciness, muscle fiber tenderness, presence of connective tissue, overall tenderness, and flavor intensity) and calving characteristics (calf vigor, calving ease, calf sex, and sire). Data collected on animals that died during the trial were not used in the statistical analyses.

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Results and DiscussionPI:

Early Weaning to Breeding

Integration of early-weaning and sexed semen into a SCH system was studied and a final economic analysis was conducted to establish profitability. In the first year 46 heifers of non-replacement status (IS heifers) were early weaned (EW) at 110 " 15.0 d of age at 141 " 21.1 kg. Forty heifers of replacement status (TRS) were traditionally weaned (TW) at 229 " 2.8 d of age at 262 " 25.40 kg. At time of TW, YI IS heifers were 202 " 15.0 d of age and weighed 249 " 6.8 kg. The YI TRS heifers were 27 " 12.2 d of age older (P < 0.01), than the YI IS heifers and had greater weights at time of TW. From EW to TW, the dams of the YI IS heifers had greater BCS than dams of YI TRS heifers, 6.6 " 0.80 and 5.8 " 0.78 (P < 0.01), as a result of lactation ending and allowing for increased biological utilization of grazing forage nutrition. Myers et al. (64) and Story et al. (97) also reported an increase in dam BCS and subsequent increased reproduction rate of 12% (64). Reproduction rate in the current study was not affected by early weaning as all dams were managed to a constant BCS prior to breeding. The dams of the YI IS heifers were put on winter range without additional supplementation under weight-loss management conditions. The dams of the TRS heifers were also put on winter range

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managed to maintain or gain BCS. During the winter period, the dams of the YI TRS heifers required very little supplementation due to the mild winter in Akron, CO in 1999-2000. Early weaning the YI IS heifers had the very minor economic benefit of \$7.06 per dam less wintering cost than the dams of the YI TRS heifers as very little supplement was needed regardless of weaning strategy.

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In year two, forty-eight YII IS heifers were weaned at a slightly older age than the first year (P < 0.01) 116 " 10.5 d of age and at a greater weight (P < 0.01) of 164 " 24.6 kg. Forty YII TRS heifers were traditionally weaned at a younger age (P < 0.01) than YI TRS heifers at 174 " 8.0 d of age due to drought conditions and the necessity to conserve range forage for winter consumption by dams. At the time of TW YII IS heifers were 25 " 5.3 d younger than YII TRS (P < 0.01) and weighed 190 " 28.9 kg. The YII TRS heifers had equal weights to YII IS heifers at this time. In contrast to YI, EW had no effect on dam BCS from EW to TW (P = 0.76) perhaps as a result of TW occurring 55 d earlier than in YII. Hence, dams of the weaned IS heifers did not have adequate time to increase BCS over the lactating dams of TRS between weaning dates. Therefore dams were not managed separately throughout the winter period.

Early weaning heifer calves in this study resulted in increased dam BCS YI as well as faster rates of gain than contemporaries that remained on dam. This is in agreement with Richardson et al. (95), Grimes and Turner (5), and Schoonmaker et al. (85). At the time of traditional-weaning YI and YII IS heifers that were early-weaned were not heavier than the YI and YII TRS heifers that were traditional-weaned as Peterson et al. (70) reported, however, weight per day of age (WDA) was greater for IS heifers than TRS heifers in YI. 25

The IS heifers 28 d weight gains throughout Phase I varied from 0.86 " 0.371 kg/d to 2.00 " 0.367 kg/d with an overall average of 1.25 " 0.139 kg/d (Figure 9) in YI. Variation in 28 d weight gains throughout PI in YII were similar to variation in YI, ranging from 0.47 " 0.581 kg/d to 2.45 " 3.804 kg/d with an overall average of 0.81 " 0.155kg/d (Figure 9). These variations in 28 d gains are attributed to adjusting feed rations to allow for gains that would induce early puberty via rapid growth of

approximately 1.3 kg/d in order to reach 65% of mature weight. Once the heifers began to cycle, the ration was decreased to avoid high BCS of heifers and possible negative impacts on subsequent reproduction/calving difficulty. Negative gains were reported in March of YII PI as a result of a limit fed diet, allowed intake to be approximately 8kg/hd/d. In addition, YII variation may also be explained by a high morbidity rate of IS heifers throughout PI.

During PI:YI, three IS heifers were taken off the study prior to breeding. One heifer died shortly after weaning due to some unknown cause, the second heifer had poor performance and negative gains, and the third heifer foundered. These losses may have been due to aggressive feeding during the early weaning period and were accounted for in the final economic analysis. Eight heifers were taken off study in the second year due entirely to death loss. Death loss of two heifers occurred shortly after weaning, attributed to respiratory disease. However, the remaining six YII IS heifers perished at various lengths into PI. Death of these heifers was attributed to and diagnosed as enterotoxaemia by a local veterinarian. The outbreak of enterotoxaemia is unexplained as all IS heifers were vaccinated for Clostridium, Type C and D and re-vaccinated after two IS heifers were lost to the disease. It may also be noted that no other feedlot cattle (including herd mates) experienced as great of a mortality or morbidity rate as result of this disease or any other disease. One explanation for high morbidity/mortality of the EW IS heifers may be due to a compromised immune system. At the time of EW, many IS heifers were sick with respiratory infections. It seems as though the IS heifers never really overcame the sickness and can be seen in ADG from EW to TW. These ADG were lower for YII IS heifers than for YI IS heifers despite similar nutritional management (Figures 1,2,3 and 4).

Puberty and Breeding

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Puberty was reached at various ages dependent on the individual heifer (Table 6). Approximately one month prior to synchronization, YI IS heifers had 20% cycling compared to YI TRS heifers at 8% (P < 0.01). The induction of early puberty of the IS heifers can be attributed to nutritional management that allowed greater gains and increased weight at this particular time (P < 0.01; 314 "28.0 kg and 293 "31.47 kg). At

the same time in relation to synchronization, fewer YII IS heifers were cycling compared to YII TRS heifers (P < 0.01; 0% and 28%) even though YII IS heifers were heavier (P<.01; 296 " 32.8 kg and 259 " 43.5 kg); however, the older age of TRS heifers can explain the percent cycling between the two groups (P < 0.01; 228 " 10.5d and 252 " 8.0 d). At this time, fewer YII IS heifers were cycling than YI IS heifers (P < 0.01), even though they were older (P < 0.01). Perhaps an explanation may be that the YI IS heifers were heavier in weight (P < 0.05). Likewise, more YII TMS heifers were cycling (P < 0.05). 0.01) at the same age (P = 0.76) but heavier weight (P < 0.01). The factors responsible for the differences in percent of heifers cycling between the two years are unknown. However, many factors varied between years such as season, diet, genetics, and social 10 environment, all of which had potential to affect timing of puberty. At the time of PGF injection the number of heifers cycling increased to 84% with 38 of 45 heifers pubertal, there was a similar (P = 0.58) increase to 75% in YII with 30 of 40 heifers pubertal. At this time YI IS heifers and YII IS heifers had similar weights (341 " 28.3 kg and 348 " 31.8 kg; P = 0.26) but YI IS heifers were younger than YII IS heifers (278 " 15.0 d and 15 291 " 10.5 d of age; P < 0.01). The YI and YII IS heifers were 68% and 70% of mature weight (assuming mature weight is 500 kg based on herd dams) at time of PGF injection. Also at AI of IS heifers, YI TRS heifers were 317 " 2.8 d and were much lighter than YI IS heifers (293 " 31.7 kg, P < 0.01). Similarly, YII TRS heifers were 316 " 8.0 d of age and were also lighter than YII IS heifers (281 " 99.6 kg, P < 0.01 YII). 20

TABLE 6. Characteristics of Integrated and Traditional Replacement System heifers prior to synchronization and at time of prostaglandin injection.									
	1 mo prior to MGA				At time of PGF injection				
†	Age	Weight	WDA	Cycling	Age	Weight	WDA	Cycling	
	(d)	(kg)	(kg/d)	(%)	(d)	(kg)	(kg/d)	(%)	
YI IS		$314 \pm 28.0^{\circ}$		20 ^b	278 ± 15.0^{a}	$341 \pm 28.3^{\circ}$	1.2 ± 0.09^{c}	81ª	
		332 ± 25.6^{d}			$314 \pm 2.8^{\circ}$	293 ± 31.7^{a}	0.9 ± 0.10^{a}	NA	
YII IS	228 ± 10.5^{b}	296 ± 32.8^{b}	$1.3 \pm 0.24^{\circ}$	Oa	291 ± 10.5^{b}	348 ± 31.8°		76ª	
YII TRS	252 ± 8.0^{c}	259 ± 43.5^{a}	$1.0\pm0.17^{\mathrm{a}}$	28 ^b	$316 \pm 8.0^{\circ}$	314 ± 20.2^{b}	1.0 ± 0.06^{b}	NA	
The different superscripts differ $(P < 0.05)$.									

Induction of early puberty of the IS heifers is attributed to the high plane of nutrition of the IS heifers. This conclusion is supported by Roux et al. (78) who reported a high plane of nutrition induced earlier onset of puberty and an increased percent of

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heifers with regular estrous cycles. Schillo et al. (83) hypothesized that nutritional status affects timing of increasing LH pulses and may involve the LH pulse generating system in the hypothalamus through an unknown mechanism. Kinder et al (44) stated that changes in body mass or fatness somehow affect LH pulses. Pre-pubertal heifers fed limited amount of energy had prolonged estradiol suppression and release of LH pulses compared to those fed a high-energy diet. High-energy diets are thought to result in larger dominant follicles earlier in life. Although plane of nutrition is inversely related to age at puberty, pattern of gain has no effect on age at puberty (33) as long as heifers reach approximately 60-65% of mature body weight (51) prior to breeding. Likewise, the pattern of gain in the current study did not affect the onset of puberty. The addition of rumensin in the diet is also thought to have hastened the onset of puberty. Randel (27) summarized several studies and found diets that had high propionate production in the rumen led to puberty at lighter weights. Similarly, Moseley et al. (62) and Purvis and Whittier (72) found that diets containing ionophores decreased the age at onset of puberty, not related to ADG or BW.

Weight of heifers seemed to have more influence than age on puberty (Table 7). The IS heifers that reached puberty at less than 9-mo of age were similar in age (P = 0.66) and weight (P = 0.15). However, the IS heifers that reached puberty at greater than 9-mo of age were of similar weight (P = 0.44) but different ages (P < 0.01). The IS heifers in YI and YII that reached puberty prior to PGF injection were of similar weight (P = 0.29) and age (P = 0.66). This supports data that weight of heifers tend to have a greater impact on the timing of puberty than age (4.78,79).

	ΥI	YII
Fotal Integrated System Heifers	43	38
Onset of Puberty		
Number of first heats	35	29
Age (d)	248 ± 31.4	313 ± 54.1
Weight (kg)	316 ± 42.9	329 ± 56.8
Onset of Puberty < 9 mo of age		
Number of first heats	. 23	6
Age (d)	233 ± 27.1	227 ± 31.3

Weight (kg)	296 ± 36.9	262 ± 82.4
Onset of Puberty > 9 mo of age		
Number of first heats	13	23
Age (d)	279 ± 6.0^{a}	335 ± 30.9^{b}
Weight (kg)	355 ± 21.4	347 ± 31.8
Onset of puberty determined by serun	n progesterone (> 1ng/m	ત્રી).
a.b Catagorical means in the same row v	with different superscrip	ts differ (P < 0.01).

Heifers that became pregnat to sexed semen on the first service of AI was 23% and 8% of those cycling and fixed-time mated, YI and YII respectively (Table 8).

Overall conception rate and pregnancy rates were 71% and 58% YI and 21% and 16% YII. The low conception rate may be due to any one or a combination of properties of semen used such as low numbers and motility of semen in each dose (3 x 10^6 YI and 6 x 10^6 YII, with at least 35% post-thaw motility). Seidel et al. (87) found low-dose sexed-semen to lower conception rate by 10-20% over normal-dose non-sexed semen. In YII pregnancy rate within each insemination was not affected by sexed semen sorted for X chromosome than non-sexed semen of the same dose (P = 0.51) for either first-service or second service (P = 0.56). Likewise, sires within YI and across YI and YII had no affect pregnancy as a result of first service (P = 0.87 and P = 0.45, YI and YII respectively). Technician also had no effect on pregnancy in YI (P = 0.93) and YII (P = 0.96).

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TABLE 8. Reproduction rates of Integrated System h and Year II.	eifers in	Year I
:	ΥI	ΥII
Integrated System heifers	43	38
Number of Integrated System heifers cycling	35	29
Fixed-time Mating		
Pregnant heifers	8	3
Pregnancy Rate of cycling heifers	23%	10%
Pregnancy Rate of all heifers	19%	8%
Post breeding season		
Pregnant heifers	25	6
Pregnancy Rate of cycling heifers	71%	21%
Pregnancy Rate of all heifers	58%	16%

Pregnancy rate in YI was not acceptable when one considers heifers were given 3 to 4 opportunities to become impregnated. Pregnancy rates in YII were very disappointing and there is no explanation for such poor performance. Perhaps the high morbidity of the heifers in YII interfered with reproductive function. Perhaps the combination of sexed-semen, low-dose insemination straws with very young breeding age of heifers may explain low pregnancy rates. Further investigation is needed to fully evaluate and draw conclusions about the mechanisms involved.

Pregnancy as a result of the first artificial insemination on a first or subsequent estrus did not have an affect on pregnancy (P = 0.97). This result is in contrast to Byerley et al. (16) whom concluded that the first estrus was less fertile than the third estrus.

In YI fetal death occurred in 4 of the 25 IS heifers bred (16%). These IS heifers experienced early fetal loss and three were rebred by natural service as a result of IS heifers commingling with the normal herd during breeding season. These IS heifers were culled from the IS and sold as bred heifers at the local auction barn in Yuma, CO. Revenue (\$650.00 per IS heifer) from these late-bred IS heifers was received by the IS and is included in the economic analysis. The forth IS heifer that experienced fetal loss did not rebreed and was kept in the IS, finished and harvested with her pregnant IS heifer counterparts.

Phase III: Parturition to Harvest

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Parturition for IS heifers was difficult, 9 of the 22 (41%) IS heifers experienced dystocia and required assistance (Table 9), of which, three required assistance of greater effort. Fifty percent of all calves born had calving ease scores of 2 or greater. Dams that gave birth to male calves experienced greater calving difficulty than dams with female progeny (P < 0.05); 56% of male calves and 44% of female calves were scored 2 or greater for calving difficulty. Dystocia problems probably stem from sire selection rather than due to heifer size or age. The two Black Angus bulls that semen was collected from in YI and packaged for AI did not differ in calving ease scores (P = 1.0). The two bulls had BW EPDs of 0.5 and 1.5 with accuracies of 0.82 and 0.37 at time of selection for this study. However, in the following breeding season the EPDs for BW for the 2 sires

increased to 2.3 and 4.1 with accuracies of 0.92 and 0.87 respectively. Birth weight did not statistically affect calving ease scores (P = 0.41), but had a positive correlation to CE (P < 0.05). It is believed that calf birth weights were the driving factor of dystocia as birth weight averaged 40 " 4.7 kg. High calving ease scores probably affects calf performance. This study showed no effect of CE on calf morbidity, ADG, or weaning weights. However, the small number of samples (20 calves) posses a problem for statistical analysis and a conclusion that CE has no effect on these factors would be misleading and

	Heifer Calves	Bull Calves	All Calves
Method of Mating Dam			
All Methods	12 (60%)	8	20
Sexed Semen (AI)	11 (69%)	. 5	16
Non-sexed Semen (AI)	0 (0%)	3	3
Natural Service	1 (100%)	0	1
Calf characteristics at Birth			
Birth weight (kg)	36 ± 10.7^{a}	40 ± 4.3^{a}	39 ± 4.6
Calving Ease ¹	1.3 ± 0.49^a	2.2 ± 0.75^{b}	1.6 ± 0.70
Calf Vigor ²	1.7 ± 1.13^{a}	1.8 ± 1.17^{a}	1.7 ± 1.13

1=no assistance; 2=assisted, easy; 3=assisted, very difficult.

Means in a row with different superscripts differ (P < 0.05).

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inaccurate.

Twelve of the 20 (60%) calves born were female (Table 9). Eleven of the 16 (69%) calves conceived from semen sorted for X-chromosome were female and all three calves conceived from non-sexed semen were bulls (100%) whereas the only calf born to natural serve was female (100%). Seidel et al. (87) reported that 86% of calves conceived from sexed semen are of the desired sex. The result of this data set of 69% of calves conceived to sexed semen were of desired sex, is not an adequate replication of their study as too few individuals were used. The low percent of desired sex was not expected as the true percent of X-chromosome sperm varied from 86-92% for the batches of semen used in the study.

²1=nursed immediately, calf health, strong; 2=nursed on own, but took some time.

The IS calves experienced 35% mortality and an additional 15% experienced morbidity. Two of the seven deaths occurred as a result of dystocia, with death occurring at or shortly after birth. Two deaths were due to accidents and the remaining three calves perished as a result of diphtheria (diagnosed by the local veterinarian). Morbidity may have resulted from inadequate calving facilities. The IS heifers calved in a dry lot pen in the feedlot. Manure management of the pen was unsatisfactory and depth of manure caused udder cleanliness to be at sub-optimal. Calving management may be one of the greatest challenges of the IS. Calving out of synchrony with other herd-mates causes labor difficulties. Additionally, calving in November through December at an operation where other feedlot animals occupy pens generate further difficulties if space is limited as well and contribute to time-management problems among employees. Weather conditions for the most part, did not affect calf mortality or morbidity as calves perished due to infectious disease rather than climatic conditions.

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The high incidence of dystocia is consistent with data reported by Boucque (11). He reported that 24 and 25-mo-old SCH Belgian White heifers bred to Charolais sires resulted in caesarean procedures on 30-37.5% and 15-25% stillbirth or death within 24 h postpartum. The high incidence of dystocia was probably due to high calf birth weight (43.8 kg and 43.9 kg). Roux et al. (78) reported similar calving difficulties in 10 mo old Friesian and Friesian X Charolais heifers bred to Aubrac and Angus sires; 39% assisted by a manager and 5% required veterinarian intervention. Incidence of calving difficulties has also reported by Bailey (5) using crossbred heifers; bred to Texas Longhorn sires less than 4% dystocia occurred, but when mated to Red Angus sires had 28% dystocia. Roux et al. (78) also found that heifers experiencing dystocia had significantly smaller pelvic measurements than heifers that calved unassisted. In the current study, no pelvic measurements were recorded in YI; however, pelvic measurements of YII IS heifers were not different to YII TRS heifers; it can be speculated that YI results would have been similar. Coleou et al. (17) reported very similar calving difficulties to the current study. In their study of Normand heifers calving at 19 mo of age, 31% required assistance, 14% experienced prenatal mortality of calves and there was 29% total mortality of calves before 3 months of age. Likewise, these calves had high birth weights of 38.5 kg.

Other researchers have also found that calf loss due to dystocia depends highly on level of supervision during calving (18). Simon et al (91) reported that under typical feedlot conditions, pregnant feedlot heifers displayed 29% calf death loss. They also reported that the high incidence of dystocia is due to a lack of facilities and unfavorable calving conditions. Nevertheless, a producer must use selection to optimize both calving ease and progeny value according to the management ability (13).

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Integrated System heifer and calf performance was acceptable in the feedlot. 10 Weaning weights of IS calves averaged 116 " 26.1 kg at 109 " 16.7 d of age and overall average daily gain of 1.0 " 0.08 kg. Average daily gains for the IS heifers varied from 1.6 " 1.03 kg during late gestation to early lactation and then decreased to 0.4 " 0.34 kg during late lactation to weaning and increased from weaning to harvest to 1.4 " 0.31 kg. Average daily gain over the 157 d PIII feeding period for IS heifers was 1.4".31 kg. The IS heifer performance was fairly constant over all heifers. This may be because a high 15 level of cross suckling occurred, so even though difference in calf age was high, each dam probably milked the same amount. Heifers that lost their calves at birth or shortly there after, continued to lactate to help support calves of other dams. Other researchers have also noted a high incidence of cross suckling among SCH rearing calves under 20 feedlot conditions (13,35). Total live-weight produced from the final twenty-two IS heifers and calves was 894 " 65.7 kg. No mortality or morbidity was experienced by the IS heifers during Phase III.

Brethour and Jaeger (13) reported that for each day delay in weaning, calf gain was 0.54 kg while gain of the dam was reduced by the equivalent amount. They therefore early weaned calves at 10-12 weeks (70-90 d) of age. The current data also found that as lactation increased to support greater calf gain, dam gain decreased. Once calves were removed, gain increased for the IS heifers. However, it must be noted that only five animals ever experienced weight loss at any point during PIII and weight loss never exceeded 0.6 kg/d with in a 28 d weigh period. This weight loss may not have been body tissue, rather, may be attributed to gut fill as animals were not fasted prior to weightgs.

Reiling et al (74) managed postpartum SCH in a feedlot on 85% concentrate diet at 13.4% CP. He noted that calves weaned at 117 d of age weighed 159 kg. Their results show better feedlot performance than the current study, however, Reiling et al. (74) weaned calves at an older age. They also reported that at the time of weaning, SCH had sub-cutaneous fat at a depth of 1.1 cm whereas, in the current study, IS heifer BF was 0.27 cm as determined by ultrasonography. In the current study, postpartum SCH were managed on 96% concentrate diet at 11.8% CP. The difference in performance between the two studies cannot be explained entirely by diet. However, varying season, genetics, and lactation ability may explain some of the difference. Brethour and Jaeger (13) found performance of SCH to be uneven, the previous comparison demonstrates variability in the system in relation to time, genetics, and management.

Phase IV: Carcass Characteristics

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The IS heifers were harvested at 715 " 15.0 d of age (24-mo) weighing 613 " 46.1 kg. Colorado State University personnel and a USDA Grader measured means and standard deviations of carcass characteristics (KPH, fat thickness, degree of marbling, ribeye area, lean maturity scores, and bone maturity scores) (Table 11). Eight of the 22 IS heifers received bone maturity score of "B." However, all had lean maturity scores of "A" (Table 10), and only four of the 8 carcasses resulted in overall "B" maturity scores. These results support the theory proposed by other researchers, that pregnancy and lactation affect bone ossification to a greater extent than lean maturity scores (Table 12). Field et al. (76) reported the most exaggerated trend; 18 head of 33-mo-old SCH had lean maturity of "A" but bone maturity "C" According to USDA (103), carcasses with "C" skeletal or lean maturity will remain "C" maturity regardless of the other physiological score. Overall maturity remained "C" maturity. Waggoner et al. (105) reported average overall carcass maturity scores of the SCH were "B" (105 " 3.0), bone maturity score of "B" (116 " 3.6), and lean maturity score of "A" (80 " 3.2) with only 3 of 84 carcasses reported as AC@ maturity. Field et al. (26) reported that parity did not affect lean firmness. However, lean color tended to be darker as animal aged from yearling maiden heifers to 2-yr old maiden heifers. Pregnancy of 30-mo old SCH resulted in lighter lean color than 2-yr old maiden heifers. Some studies reported maiden females had greater

maturity scores in lean color (5,74,104,105). Hermesmeyer et al. (35) reported that lactating SCH had greater lean maturity scores than did open heifers of the same age, but bone maturity scores did not differ. Therefore, overall maturity scores were greater for SCH than open heifers. In contrast, other research data found no difference between lean color of SCH and maiden heifers (10,14,26,78). In the current study, effect of pregnancy and lactation as separate factors on bone and lean maturity could not be accessed as the IS heifers that lost their calves continued to lactate as a result of cross-suckling.

	Bone	Lean									Calcu	lated	USDA	Grade
Animal ID	Maturity		Overall Maturtiy ^a	Degree of Marbling ^b	HCW (kg)	PYG	Adjusted PYG	Adjusted Fat (cm)	REA (cm²)	KPH (%)	Quality Grade	Yield Grade	Quality Grade	Yield Grad
152	70	60	65	390	724	3.3	3,3	0.52	13.3	1.5	Ch	2.60	Ch	3
155	90	100	95	610	756	2.5	2.6	0.24	11.4	1.5	Ch	2.62	Ch	2
108	80	80	80	570	762	3	3.1	0.44	13.1	2	Ch	2.70	Ch	2
110	80	100	90	410	746	3	3.2	0.48	13	2	Ch	2.77	Ch	2
167	130	60	95	460	888	3.3	3.3	0.52	14.9	2	Ch	2.81	Ch	3
165	60	80	70	360	791	3.5	3.3	0.52	13.5	2	Ch	2.89	Ch	2
179	110	70	90	480	929	3.5	3.5	0.6	15.4	2	. Ch	3.00	Ch	3
192	70	70	70	420	777	3.5	3.6	0.64	14	2.5	Ch	3.07	Ch	3
134	60	70	65	460	821	3.2	3.3	0.52	13.3	2.5	Ch	3.16	Ch	3
151	70	70	70	500	820	3,3	3.3	0.52	12.9	2.5	Ch	3.29	Ch	3
186	120	50	85	520	828	3.3	3.4	0.56	13.1	2.5	Ch	3.35	Ch	3
131	180	90	135	410	848	3.8	3.9	0.76	14.8	2.5	Ch	3.39	Ch	3
127	80	110	95	430	848	3.3	3.4	0.56	11.5	1.5	Cb	3.74	Ch	3
180	60	80	70	520	733	3.7	3.7	0.68	11.6	2.5	Ch	3.77	Ch	3
195	70	70	70	440	766	4	4	0.8	12	2	Ch	3.97	Ch	3
137	170	60	115	450	911	4.3	4.3	0.92	13.7	2	Ch	4.28	Ch	3
177	140	60	100	500	836	4.8	4.8	1.12	14.5	2.5	Ch	4.34	Ch	4
138	80	70	75	340	751	2.7	2.7	0.28	15.3	1	Se	1.36	Se	2
213	70	90	80	380	698	2.7	2.8	0.32	12.5	1	Se	2.15	Se	2
163	60	70	65	350	760	2.8	3	0.4	12.2	2	Se	2.88	Se	2
143	180	80	130	380°	778	3	3.3	0.52	14.4	2	St	2.55	Se	2
205	160	60	110	430°	737	5	3	0.4	11.9	2	St	2.89	Ch	2

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The IS heifers averaged Small degree of marbling (Table 11). Of the 17 carcasses that received overall maturity scores of "A", 14 carcasses received Choice quality grades, and 3 carcasses received Select quality grades (Table 10). Of the 5 carcasses that

received overall carcass maturity score "B", 1 of the carcasses had enough marbling (Modest) to remain in the Choice quality grade. The other 4 carcasses received Standard quality grade as a result of Small degree of marbling. These four carcasses received financial discounts of \$11.87 per cwt (Table 5). Other discounts received included 9 of the 22 carcasses with YG of 3 at \$1.00/cwt and 2 of the 22 carcasses with YG of 4 at \$20.00/cwt. Consequently, one the carcasses that received YG 4 was also a "B" maturity carcasses. One of the 2 YG 4 had the minimum degree marbling to remain in Choice quality grade. However, since the discount for YG 4 is greater than for a Standard carcass, one should not keep animals on feed too long to attempt to increase intramuscular fat in order to overcome "B" maturity carcasses resulting in Standard grade. Furthermore the other carcasses that received YG 4 also received Standard quality grade and the discount for this particular carcass was \$36.87/cwt.

_	n	HCW (kg)	Dressing (%)	Bone Maturity ^a	Lean Maturity*	Overall Maturity	Marbling ^b	Adj. Fat (cm)	REA (cm²)	КРН	Quality Grade	Yield Grade
"A" Maturity	17	357 ± 27.5	60 ± 2.5	80 ± 21.2	76 ± 15.8	78 ± 1.3	449 ± 77 i	1 28 ± 0 361	84.64 ± 7.991	1.94 ±0 496	Small ⁵⁰	2.95 ± 0.623
"B"	••	057-2712										
Maturity All	5	373 ± 30.2	61 ± 0.6	166 ± 8.8	70 ± 13.0	118 ± 10.5	434 ± 26.9	1.89± 0.526	89.42 ± 7.185	2,20 ± 0,219	Small ³⁰	3.49 ± 0.657
	22	361 ± 28.2	60 ± 2.2	100 ± 41.9	75 ±15.4	87 ± 20.7	446 ± 70.4	1.42 ± 0.521	85.72 ± 7.980	2.00 ± 0.463	Small ⁵⁰	3.07 ± 0.687

The IS heifers deposited intramuscular adequately as most were of Choice quality grade. However, due to the influence of "B" maturity, 4 carcasses were graded Standard. The occurance of Standard carcasses are of major economical concern as these carcasses were discounted by \$11.87/cwt under the base price and Choice carcasses were given premiums of only \$0.97/cwt over the base price (Table 5). With this particular grid, it would take about 12 Choice carcasses to compensate for the discounts of a single Standard carcass of the same Yield Grade and weight. The USDA (103) made a change to the role of which B maturity plays in quality grades. Currently, B-maturity carcasses with less than modest degree of marbling will be graded standard; those carcasses with at least modest degree of marbling will receive the Choice grade or better. The new grading standard may increase the incidence of heiferetts graded Standard (75) from studies conducted on SCH studies in the past. Standard grade of a carcass is associated with discounts and may be a major economic impact on profitability of feeding SCH

(26,74,100,105).

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In the current study, only 1 of the 4 "B" maturity carcasses remained in Choice quality grade due to Modest degree of marbling, whereas before the grade standard change, all 4 carcasses would have been graded Choice. Likewise, many previous studies that reported a high incidence of Choice carcasses would now result in lower grade of Standard. Hermesmeyer et al. (36) reported that 74% of the SCH received USDA Choice, YG 3. Likewise, Reiling et al. (75) reported that approximately 50% of SCH would have received USDA Choice under the old USDA Grade Standards (102). However, under current USDA Standards (103) the percent of heiferetts that would receive the same grade would decrease to about 40%.

The results of this study are similar to the carcass characteristics found by many other SCH system studies (Table 12), even though animals were 24-mo of age compared to 30-mo old heifers. However, several studies have reported some carcass maturity scores of "C" (26,74,75,105). In the current study, no incidence of "C" maturity occurred. Similarly, Brethour and Jaegar (13) and Waggoner (105) found no carcasses with carcass maturity scores of "C".

	n	Age (mo)	Bone Maturity	Lean Maturity ^a	Overall Maturity ^a	Marblingb	Yield Grade
Field et al. (1997) Early Weaned Calves (120 d)		33.2	202	59	200	390	3.30
Waggoner et al. (1990) Early Weaned Calves (115 d)	52	30	116	80	105	500	2.00
Hermesmeyer (1999a)				*-		-	
Exp 1. Lactating	6	28	119	111	115	449	3.05
Exp 2. Early Weaned Calves (64 d, 89 d)	12	30	117	104	113	423	2.45
Exp 2. Lactating	10	30	145	141	143	407	2.89
Reiling et al. (1995) Early Weaned Calves (117 d)		27	98	68	82	475	3.08
Reiling et al. (1996)	29		,,,	•	02	475	2.00
Exp 2. Early Weaned Calves (111 d)	23	32	139	120	138	415	2.27
Exp 2. Early Weaned Calves (111 d)	23	32	125	114	125	426	2.27
Exp 3. Normal Weaned Calves (211 d)	25	32	97	91	98	472	2.47
Exp 3. Normal Weaned Calves (211 d)	26	32	112	103	110	450	2.61
0-99=A°-A°°; 100-199=B°-B°°; 200-299=C°-C°		32	•••	103		-150	2.01

Warner-Bratzler Shear Force and Sensory Taste Panel

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Waggoner et al. (105), Joseph and Crowley (42), and Bond et al. (10) reported that juiciness and flavor was not affected by parity nor was sensory panel tenderness. Waggoner et al. (105) reported that sensory panelists found detectable connective tissue, myofibrillar and overall tenderness to be higher for yearling maiden heifers than either SCH or 2 yr-old maiden heifers. The WBS values were higher for SCH than maiden heifers. Therefore, calving had a negative affect on tenderness. Age increased sensory panel detectable connective tissue and the combined affect of age and parturition decreased tenderness over yearling maiden heifers. However, tenderness and palatability traits did not differ between 2 yr-old maiden heifers and SCH. Therefore, the SCH system resulted in meat palatability comparable to maiden heifers of a similar age as determined by sensory panelists. Vincent et al. (104) reported SCH did not differ in sensory panel ratings except for the oldest (33 mo of age) SCH, which had greater connective tissue. Joseph and Crowley (42) finished Hereford crossbred maiden heifers and SCH on pasture and reported that calved heifers appeared to be as acceptable to sensory panelists as maiden heifers and both were nearly as acceptable as steers. There was no significant difference in tenderness, juiciness or flavor between maiden heifers and SCH heifers. In the current study, 8 panelist rated the meat from the IS heifers quite acceptable. On an 8-point scale, panelists rated the steaks moderately juicy (6 " 0.99), moderate in muscle fiber tenderness (6 " 1.1), trace amount of connective tissue present (6 " 1.1), moderate in overall tenderness (6 " 0.95) and a flavor that is slightly detectable (1 " 0.61).

Classification of carcasses maturity as "A" or "B" did not affect any of the tastepanel characteristics evaluated (P > 0.3). Although increasing carcass maturity is
associated with a decrease in tenderness, juiciness and an increase in flavor intensity and
off flavors, Hilton et al. (37) similarly found position within a maturity group had a
negligible effect on palatability. Likewise, other researchers have reported sensory taste
panel traits were undifferentiated between "A" and "B" maturity carcasses (99). In
contrast to these studies and the current study, Smith et al. (93,94) reported steaks from
"A" and "B" maturity carcasses, within equal marbling scores, were significantly
different. The correlation between "A" and "B" maturity classifications are not clear and

further research studies have provided evidence that has contributed to the confusion of the discrepancy between "A" and "B" maturity classifications and palatability. However, the combined effects of marbling and maturity, accounts for 30-40% of the observed variation in tenderness, 26% of the variation in juiciness, and 15% of variation in flavor (37).

All 22 steaks from the IS heifers were tender. The average Warner-Bratzler Shear force (WBS) value was 2.9 " 0.9. No incidence of tough steaks occurred in the current study as no steak exceeded WBS value of 4.5 (89). It is therefore concluded that steaks for 24-mo old SCH managed in the IS provide consumers with a highly palatable product.

Phase V: Economic Analysis

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Economic analysis of each phase was conducted to determine net revenue/loss. Phase I (Table 13) of YI had a net loss of \$28,800.49, due mainly to investment of IS heifers (\$15,540.64) and 3 cows for androgenization (\$1,470.00). Breeding was the second most costly expenditure at \$3,210.74 followed by feed costs (\$8,697.39). Revenue (\$2,591.48) in Phase I was generated by cull androgenized cows and IS heifers due to poor feedlot performance (realizers).

TABLE 13. Inc	come Statement fo	r Year I Phas	e I.	
REVENUES	# Head	Ave WT	Market Price	Total
Cull Androgenized Cow	3	16.44	\$44.00	\$2,170.08
Realizers	2	4.9	\$43.00	\$421.40
TOTAL				\$2,591.48
GROSS INCOME				\$2,591.48
EXPENSES				
Livestock	# Head		Market Price	Total
IS Heifers	46	3.28		\$15,540.64
Androgenized Cow	3	14		\$1,470.00
Death Loss of IS Heifer	1	3.28	\$103.00	\$337.84
TOTAL				\$17,348.48
Feed	# Head	Intake (ton)	Price/ton	Total
Corn	46	56.375	\$70.00	\$3,946.25
Alfalfa Hay		17.882	\$55.00	\$983.51
Medicated Feed		0.088	\$265.13	\$23.33
Supplement 517		13.372	\$185.00	\$2,473.82

Markup		84.6987	\$15.00	\$1,270.48
TOTAL		172.4157	\$590.13	\$8,697.39
Yardage	# Head	Rate	# Head Days	Total
Head In	43	\$0.20	9988	\$1,997.60
Dead .	1	\$0.20	35	\$7.0
Realized	2	\$0.20	340	\$68.00
TOTAL	46		10363	\$2,072.60
 Health	# Head	Rate		Total
Processing	48	\$1.00		\$48.00
Hospital drugs		\$10.76	,	\$10.76
Chute Charge	4	\$1.00	1	\$4.0
TOTAL				\$62.76
Breeding Cost	# Head	Rate	,	Total
MGA	43	\$3.08	3	\$132.44
PGF	43	\$2.10)	\$90.30
Semen Sorting Fee	83	\$20.00)	\$1,660.00
Semen Dose	83	\$12.00)	\$996.00
Technician Fee	83	\$4.00)	\$332.00
TOTAL				\$3,210.74
TOTAL OPERATING EXPENSE	•			\$31,391.97
NET REVENUE/LOSS (Income-expenses)		٠.		(\$28.800.49)

Phase II (Table 14) revenue was generated by sale of 18 IS heifers that had failed to get pregnant. These heifers were sold immediately after the final pregnancy check for \$11,880.00. The expenses totaled \$1,860.69. Pasture lease was \$13.00 per AUM and each IS heifer was considered .65 AUM. The 18 open IS heifers remained in pasture with their pregnant counterparts for about 1 2 mo. The 25 pregnant IS heifers remained in pasture for about 6 2 mo. Net revenue for PII was \$10,019.31. Phase III (Table 15) had a net revenue of \$18,866.02. Major expenses included death loss of calves from the IS heifers (\$2,415.60) and feed costs of \$6,206.31. The major source of revenue was created by 22 IS heifers that were fed to finish and harvested. These heifers were marketed according to carcass merit (Table 5). Revenue created (\$21,510.53) by the IS heifers are categorized according to carcass merit in Table 16.

The IS was not more profitable than traditional management system of nonreplacement heifers in which heifer calves are weaned at a traditional age of 200 d and sold immediately after weaning (Table 17). The gross revenue of the TMS was \$21,834.80 generated by sale of 43 TW calves at \$88.00/lb. Expense of the TMS was

cow cost. Cow cost was calculated by taking the latest 5-year cow costs at ECRC and averaging (\$19,352.58) then multiplying by 43 TMS calves. The difference between the IS and the TMS was \$3,944.67 in favor of the TMS. However, in the simulations (Table 18) at 58% pregnancy rate, calf survival increased the difference to \$3,679.36 in favor of the IS. This result demonstrates the economic importance of calf survival. By decreasing the number of calves that died from 27% to 2%, revenue increased by \$7,624.03. It is therefore evident that calving management must take a priority in the final phase of the system. As stated previously, this period of time and level of supervision required may cause labor management problems. Hence, individual producers should evaluate adoption of this system according to management abilities.

The simulations (Table 18) showed that under the assumptions, the IS can be profitable above the TMS. The most appropriate figures to assess the system are those associated with 80% reproduction rate. This rate is the most acceptable pregnancy rate and could probably be achieve with the IS with further development and research into the project. At this pregnancy rate revenue above the TMS would be \$7,321.37. However, achieving this pregnancy rate may or may not be accomplished. The simulations did not include wages for labor during the calving season nor did it include interest rates or opportunity costs. The simulations reported in this study should therefore be interpreted with caution, as profitability would certainly fall if these factors were included.

Implications

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The integrated system in which early-weaning and sexed-semen are incorporated into the single-calf heifer system, is an accelerated system that allows one calf to be born to a heifer targeted for slaughter at 24-mo of age. The IS depends on achieving an early puberty allowing a IS heifer to be bred at 10-mo of age. A strong limiting factor of the system is the ability or the inability of a heifer at that age to become pregnant. Consumer satisfaction will not be jeopardized by meat provided by IS heifers as the end product is highly palatable to a taste-panel and very tender according to Warner-Bratzler Shear force. If a high reproduction rate can be achieved and calf mortality kept to a minimum, great profitability can be achieved over selling non-replacement heifers immediately following weaning at a traditional age. Although the IS has potential to be profitable and

provide quality end product to the consumer, adoption of the system by an operation should be assessed on an individual basis according to managerial ability.

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The discussion included in this Patent Cooperation Treaty patent application is intended to serve as a basic description of the invention. The reader should be aware that the specific discussion may not explicitly describe all the embodiments of the invention that are possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in functionally-oriented terminology, each aspect of the function can be accomplished by a device, subroutine, or program. Apparatus claims may not only be included for the devices described, but also method or process claims may be included to address the functions the inventions and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. This disclosure should be understood to encompass each such variation, be it a variation of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element that causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of an "estrous synchronization element" should be understood to encompass disclosure of the act of "synchronizing estrous" -- whether explicitly discussed or not -- and, conversely, were there only disclosure of the act of

"synchronizing estrous", such a disclosure should be understood to encompass disclosure of a "estrous synchronization element" and even a means for "synchronizing estrous". Such changes and alternative terms are to be understood to be explicitly included in the description.

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Additionally, various combinations and permutations of all elements of applications can be created and presented. All can be done to optimize the design or performance in specific applications.

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Any acts of law, statutes, regulations, or rules mentioned in this application for patent; or patent, publications, or other references mentioned in this application for patent are hereby incorporated by reference herein. Specifically, United States Provisional Patent Application Nos. 60/211093, filed on June 12, 2000 and 60/224,050, filed on August 9, 2000, and United States Patent Application No. 09/001,394, filed on December 31, 1997 and United States Patent Application No. 09/015,454, filed January 29, 1998 and PCT/US99/17165, filed July 28, 1999, and "Cost Effectiveness of Utilizing Sexed-Semen in a Commercial Beef Cow Operation," thesis of Nanette Lynn Steel, Department of Agriculture and Resource Economics, Summer of 1998 are each hereby incorporated by reference.

DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	FILING DATE
32,350	02/10/87	Bhattacharya	204	180.1	11/22/74
3,687,806	08/29/72	Van den Bovenkamp	195	1.3	11/04/69
3,829,216	08/13/74	Persidsky	356	36	
3,894,529	07/15/75	Shrimpton	128	1 R	04/10/69
4,009,260	02/22/77	Ericsson	424	105	12/11/74
4,067,965	01/10/78	Bhattacharya	424	105	12/17/75
4,083,957	04/11/78	Lang	424	78	02/04/76
4,085,205	04/18/78	Hancock	424	105	01/24/77
4,092,229	05/30/78	Bhattacharya	204	180 R	10/20/76
4,155,831	05/22/79	Bhattacharya	207	299 R	02/23/78
4,191,749	03/04/80	Bryant	424	105	10/11/77
4,225,405	09/30/80	Lawson	204	180 R	08/16/78

4,276,139	06/30/81	Lawson	204	180 R	10/09/79
4,339,434	07/13/82	Ericsson	424	105	08/17/81
4,362,246	12/07/82	Adair	209	3.3	07/14/80
4,448,767	05/15/84	Bryant	424	85	02/15/80
4,501,366	02/26/85	Thompson	209	556	12/14/82
4,511,661	04/16/85	Goldberg	436	503	12/30/83
4,660,971	04/28/87	Sage et al.	356	39	05/03/84
4,680,258	07/14/87	Hammerling et al	435	7	08/09/83
4,680,258	07/14/87	Hammerling et al	435	7	08/09/83
4,698,142	10/06/87	Muroi et al	204	182.3	07/31/85
4,749,458	06/07/88	Muroi et al	204	182.3	03/02/87
4,988,619	01/29/91	Pinkel	435	30	11/30/87
4,999,283	03/12/91	Zavos et al	435	2	08/18/89
5,021,244	06/04/91	Spaulding	424	561	05/12/89
5,135,759	08/04/92	Johnson	424	561	04/26/91
5,346,990	09/13/94	Spaulding	530	350	03/12/91
5,371,585	12/06/94	Morgan et al.	356	246	11/10/92
5,439,362	08/08/95	Spaulding	424	185.1	07/25/94
5,466,572	11/14/95	Sasaki et al.	435	2	04/25/94
5,483,469	01/09/96	Van den Engh et al.	364	555	08/02/93
5,503,994	04/02/96	Shear et al.	436	90	10/08/93
5,514,537	05/07/96	Chandler	435	2	11/28/94
5,589,457	12/31/96	Wiltbank	514	12	07/03/95
5,602,039	02/11/97	Van den Engh	436	164	10/14/94
5,602,349	02/11/97	Van den Engh	73	864.85	10/14/94
5,660,997	08/26/97	Spaulding	435	7.21	06/07/95
5,690,895	11/25/97	Matsumoto et al.	422	73	12/06/96
5,700,692	12/23/97	Sweet	436	50	09/27/94
5,726,364	03/10/98	Van den Engh	73	864.85	02/10/97
5,880,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,985,216	11/16/99	Rens et al.	 		07/24/97
6,071,689	06/06/00	Seidel et al.	435	2	01/29/98
0,071,007	00,00,00				

DOCUMENT NO	DATE	COUNTRY	CLASS	SUBCLASS
WO 96/12171	10/13/95	United States	6	
WO 98/34094	06/08/98	New Zealand		

WO 99/05504	07/24/98	United States	
WO 99/33956	08/07/99	United States	
WO 99/38883	05/08/99	United States	
WO 99/42810	26/08/99	United States	
WO 00/06193	10/02/00	United States	

Akhtar, S., et al., "Prevalence of Five Stereotypes of Bluetongue Virus in a Rambouillet Sheep Flock in Pakistan", Veterinary Record 136, 1995, p. 495.

Akhtar, S., et al., "Sex Preselected in Cattle: a Field Trial", Veterinary Record 136, 1995, p. 495-496.

Aldrich, S. L., Berger, L.L., Reiling, B.A., Kegler, D.I., and Nagh, T.G.. 1995. "Parturition and periparturient reproductive and metabolic hormone concentration in prenatally androgenized beefheifer", I. Anim. Sci. 73:3712.

Amann, R. P. "Issues affecting commercialization of sexed sperm". Therio: 52:1441, 1999

Amann, R.P. et al, "Prospects For Sexing Mammalian Sperm," Colorado Associated University Press, Animal Reproduction Laboratory College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523, 1982

American Meat Science Association in cooperation with National Livestock and Meat Board. "Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meatK", 1995

Amoah, E.A. and Gelaye, S. 1996. Biotechnological advances in goat reproduction. J. Anim. Sci. 75(2):578-585.

Andersen, V.K., Aamdal, J. and Fougner, J.A. 1973. Intrauterine und tiefzervikale Insemination mit Gefriersperma bein Schat. Zuchthygiene. 8:113-118.

"Applying Semen Sexing Technology to the AI Industry", National Association of Animal Breeders, September 2000, pp. 1-16

Bagley, C. P. 1993. Nutritional management of replacement beef heifers -A review. J. Anim. Sci. 71:3155-3163.

Bailey, C. M., Reid, C.R., Ringkob, T.P., Koh, Y.O., and Foote, W.D. "Nulliparous versus primiparous crossbred females for beef." J. Anim. Sci. 69:1403., 1991

Baker, R.D., Dziuk, P.J. and Norton, H.W. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci. 27:88-93.

Barnes, F.L. and Eyestone, W.H., "Early Cleavage and the Maternal Zygotic Transition in Bovine Embryos", Theriogenology, Vol. 33, No. 1, January 1990, pp. 141-149

Becker, S.E. and Johnson, A.L. 1992. Effects of gonadotropin releasing hormone infused in a pulsatite or continuous fashion on serum gonadotropin concentrations and ovulation in the mare. J. Anim. Sci. 70:1208-1215.

Bedford, S. J. and Hinrichs, K. 1994. The effect of insemination volume on pregnancy rates of pony mares. Theriogenology 42:571-578.

Bellows, R. A., Short, R.E., Anderson, D.C., Knapp, B.W., and Pahnish, O.F. "Cause and effect relationships associated with calving difficulty and calfbirth weight", J. Anim. Sci. 33:407, 1971

Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K.lnskeep. "Sourceof progesterolle prior to puberty in beef heifers". J. Anim. Sci. 49:1276., 1979

Berger, G.S. 1987. Intratubal insemination. Fert. Steril. 48:328-330.

Bergfeld, E. G., Kojima, F.N., Cupp, A.S., Wehnnan, M.E., Peters, K.T., Garciawinder, M., and Kinder, J.E., "Ovarian follicular development in prepubertal heifers is influenced by level of dietary energy-intake", Bio. of Repro. 51:1051, 1994

Berry, B. W., Smith, G.C., and Carpente.zl, "Beef carcass maturity indicators and palatability attributes", J. Anim. Sci. 38:507, 1974

Beyhan, Z., et al., "Sexual Dimorphism in IVM-IVF Bovine Embryos Produced from X and Y Chromosome-bearing Spermatozoa Sorted by High Speed Flow Cytometry", Theriogenology 52, 1999, pp. 35-48.

Blanchard, T. and Dickson, V., Stallion Management, The Veterinary Clinics of North America, Equine Practice, Vol. 8, No. 1, April 1992, pp 207 - 218.

Bond, J., et al., "Growth and carcass traits of open beef heifers versus beef heifers that have calved", Nutrition Reports International 34:621. 1986

Boucque, C. V., et al., "Beef-production with maiden and once-calved heifers", Livestock Prod. Sci. 7:121. 1980

Bourdon, R. M. and J. S. Brinks. "Simulated efficiency of range beef-production". Culling strategies and nontraditional management-systems. J. Anim. Sci.65:963. 1987

Bracher, V. and Allen, W.R., "Videoendoscopic Examination of the Mare□s Uterus: Findings in Normal Fertile Mares", Equine Veterinary Journal, Vol. 24 (1992), pp. 274-278

Braselton, W.E. and McShan, W.H. 1970. Purification and properties of follicle stimulating and luteinizing hormones from horse pituitary glands. Arch. Biochem. Biophys. 139:45-48.

Brethour, J. R., "The single-calfheifer system", Kans. Agric. Sta. Rep. Frog. 570. 1989

Brethour, J.R. and Jaeger, J.R., "The Single Calf Heifer System", Kansas Agric. Sta. Rep of Progress 570, 1989.

Bristol, S.P. 1982. Breeding behavior of a stallion at pasture with 20 mares in synchronized oestrus. J. Reprod. Fert. Suppl. 32:71.

Brookes, A. J. and Obyme, M., "Use of cow-heifers in beef production", J. of the Royal Agricultural Society of England 126:30. 1965

Buchanan, B.R., et al, "Insemination of Mares with Low Numbers of Either Unsexed or Sexed Spermatozoa", Theriogenology, Vol. 53, pp 1333-1344, (2000)

Burns, P. D. and Spitzer, J.C., "Influence ofbiostimulation on reproduction in postpartum beef-cows", J. Anim. Sci. 70:358. 1992

Burwash, L.D., Pickett, B.W., Voss, J.L. and Back, D.G. 1974. Relatioship of duration of estms to pregnancy rate in normally cycling, non-lactating mares. J.A.V.M.A. 165:714-716.

Byerley, D. J., et al., "Pregnancy rates of beef heifers bred either on puberal or 3rd estrus". J Anim. Sci. 65:645. 1987

Caslick, E.A., "The Vulva and the Vulvo-vaginal Orifice and its Relation to Genital Health of the Thoroughbred Mare" Cornell Veterinarian, Vol. 27, 1937, pp. 178-187

Catt, et al., "Assessment of Ram and Boar Spermatozoa During Cell-Sorting by Flow Cytometry□" Reproduction Dom Animal, Vol. 32, 1997, pp 251-258.

Catt, S.L., et al., 'Birth of a Male Lamb Derived from an In Vitro Matured Oocyte Fertilized by Intracytoplasmic Injection of a Single Presumptive Male Sperm", Veterinary Record 139, 1996, pp. 494-495.

Cattell, et al., "Assessment of Ram and Boar Spermatozoa During Cell-Sorting by Flow Cytometry", Reproduction Dom Animal, Vol. 32, pp 251-258 (1997)

Chin, W.W. and Boime, I. 1990. In: Glycoprotein Hormones. Serona Symp. Norwell, MA. pp. 19-20

Chung, Y.G., Schenk, J.L., Herickhoff, L.A. and Seidel, G.E. Jr. 1998. Artificial insemination of superoyulated heifers with 600,000 sexed sperm. J Anim. Sci. Suppl. 1. 836:215. abstr.

Clement, F., Vincent, P., Mahla, R., Meriaux, J.C. and Palmer, E. 1998. Which insemination fertilizes when several successive inseminations are performed before ovulation. 7th Int. Symp. Eq. Repro. 151. abstr

Coleou, J., et al., "Essai de velage tres precoce de genisses en vue de la production de viande." Essai

Vauz/ Aure no.50, programme USFGC-INAPG-ITFC. 1974

Cran, D.G., et al, "Production of Lambs by Low Dose Intrauterine Insemination with Flow Cytometrically Sorted and Unsorted Semen", Theriogenology, Vol. 47, pp. 267, (Abstract), (1997)

Cran, D.G., et al., "Production of Bovine Calves Following Separation of X- and Y- Chromosome Bearing Sperm and In Vitro Fertilisation", Veterinary Record 132, 1993, pp. 40-41.

Crowley, J. P. The facts of once-bred heifer production. (Ed) J.B. Owens. The maiden female-a means of increasing meat production. School of Agric., Univ. of Aberdeen, Scotland. 1973

Curran, S. 1998. In: Equine Diagnostic Ultrasonography. Fetal gender determination. Rantanen & McKinnon. 1st Ed. Williams and Wilkins. pp. 165-169.

Day, B.N., Abeydeera, L.R., Johnson, L.A., Welch, G.R., Wang, W.H., Cantley, T.C. and Rieke, A. 1998. Birth of piglets preselected for gender following in vitro fertilization of in vitro matured pig oocytes by X and Y bearing spermatozoa sorted by high speed flow cytometry. Theriogenology. 49(1):360. abstr.

Dean, P.N., Pinkel, D. and Mendelsob. n, M.L. 1978. Hydrodynamic orientation of spermatozoa heads for flow cytometry. Biophys. J. 23:7-13.

Demick, D.S., Voss, J.L. and Pickett, B.W. 1976. Effect of cooling, storage, glycerization and spermatozoal numbers on equine fertility. J. Anim. Sci. 43:633-637.

DenDaas, J.H.G., De Jong, G., Lansbergen, L.M.T.E. and Van Wagtendonk-De Leeuw, A.M. 1998. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of dairy bulls. J Dairy Sci. 81: 1714-1723.

Denham, A. "In-vitro studies on sandhill range forage as related to cattle preference", M.S. Thesis. 1965. Colorado State University.

Deutscher, G. H. "Extending interval from seventeen to nineteen days in the melengestrol acetate-prostaglandin estrous synchronization program for heifers". The Professional Animal Scientist 16:164, 2000

Diagnostic Products Corporation. Coat-A-Count@ Progesterone.com. 1998.

Dikeman, M. E. "Cattle production systems to meet future consumer demands. J. Anim. Sci. 59:1631, 1984

Dinnyes, A., et al., "Timing of the First Cleavage Post-insemination Affects Cryosurvival of In Vitro-produced Bovine Blastocysts", Molec Reprod develop 53, 1999, pp 318-324.

Donaldson, L. E., "Effect of Insemination Regimen on Embryo Production in Superovulated Cows", The Veterinary Record, July 13, 1985, pp. 35-37

Donoghue, A.M., Byers, A.P., Johnston, L.A., Armstrong, D.L. and Wildt, D.E. 1996. Timing of ovulation after gonadotropin induction and its importance to successful intrauterine insemination in the tiger (Panthera tigris). J. Reprod. Fert. 107:53-58.

Douglas, R.H., Nuti, L. and Ginther, O.J. 1974. Induction of ovulation and multiple ovulation on seasonally-anovulatory mares with equine pituitary fractions. Theriogenology. 2(6): 133-142.

Douglas, R.H. 1979. Review of superovulation and embryo transfer in the equine. Theriogenology. 11:33-46.

Doyle, S. P., et al. "Artificial insemination of lactating angus cows with sexed semen". Proc. Western Sect. Am.Soc.Anim. Sci. 50:203. 1999

Duchamp, G., Bour, B., Combamous, Y. and Palmer, E. 1987. Alternative solutions to hCG induction of ovulation in the mare. J. Reprod. Fert. Suppl. 35:221-228.

Evans, M.J. and Irvine, C.H.G. 1977. Induction of follicular development, maturation and ovulation by gonadotropin releasing hormone administration to acyclic mares. Bio. Reprod. 16:452-462.

Ferrell, C. L. and T. G. Jenkins. "Energy-Utilization by Mature, nonpregnant, nonlactating cows of different types" J. Anim. Sci. 58:234. 1984

Ferrell, C. L. "Effects of post-weaning rate of gain on onset of puberty and productive performance of heifers of different breeds. J. Anim. Sci. 55:1272. 1982

Field, R. A., et al., "Bone-ossification and carcass characteristics of wethers given silastic implants containing estradiol". I. Anim. Sci. 68:3663-3668. 1990

Field, R., R. et al., "Growth, carcass, and tenderness characteristics of virgin, spayed, and single-calfheifers.", J. Anim. Sci. 74:2178. 1996

Fitzgerald, B.P., Peterson, K.D. and Silvia, P.J. 1993. Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: Preliminary evidence on suppression of oyulation during the breeding season. Am. J. Vet. Res. 54:1746-1751.

Fluharty, F. L., et al., "Effect of weaning and diet on growth of calves." Research and Reviews. The Ohio State University Department of Animal Sciences. 1996

Fluharty, F.L., et al., "Effects of Age at Weaning and Diet on Growth of Calves", Ohio Agri. Res. and Dev. Circular, 1996, 156: 29.

Foulkes, J.A., Stewart, D.L. and Herbert, C.N. 1977. Artificial insemination of cattle using varying numbers of spermatozoa. Vet. Rec. 101:205.

Fugger, E.F., "Clinical Experience with Flow Cytometric Separation of Human X- and Y- Chromosome Bearing Sperm", Theriogenology, Vol. 52, pp. 1435-1440 (1999)

Fulwyler, M.J. 1977. Hydrodynamic orientation of cells. J Histochem. Cytochem. 25:781-783.

Fulwyler, M.J. 1965. Electronic separation of biological cells by volume. Science. 150:910.

G.E. Seidel, Jr., "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Diary, Loveland, CO, USA January 1996.

Garner, D.L., Gledhill, B.L., Pinkel, D., Lake, S., Stephenson, D., Van Dilla, M.A. and Johnson, L.A. 1983. Quanti-cation of the X and Y chromosome-bearing spermatozoa of domestic animals by flow cytometry. Biol. Reprod. 28:312-321.

Ginther, O.J. 1983. Sexual behavior following introduction of a stallion into a group of mares. Theriogenology. 19:877.

Ginther, O.J. 1992. In: Reproductive Biology of the Mare. (2nd Ed.) Equiservices, Cross Plains, WI.

Gledhill, B.L. 1988. Gender preselection: historical, technical and ethical perspective. Semin Reprod. Endocrinol. 6:385-395.

Gombe, S. and Hansel, W. "Plasma luteinizing-hormone (LH) and progesterone levels in heifers on restricted energy intakes." J. Anim. Sci. 37:728. 1973

Gourley, D.D. and Riese, R.L. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. N. Amer: Food Anim. Prac. 6(3):615-633.

Gravert, H. 0., "Genetic Aspects of Early Calving." In: J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 59. 1975

Gregory, K. E., et al., "Characterization of biological types of cattle III .2." Growth-rate and puberty in females. J. Anim. Sci. 49:461. 1979

Grimes, I. F, and T. B. Turner. "Early weaning of fall born calves II." Post weaning performance of early and normal-weaned calves. I. Prod. Agric. 4:168. 1991

Grondahl, C., et al, "In Vitro Production of Equine Embryos", Biology of Reproduction, Monograph Series I, pp. 299-307 (1995)

Guillou, F. and Combamous, Y. 1983. Purification of equine gonadotropins and comparative study of their acid-dissociation and receptor-binding specificity. Biochem. Biophys. Acta. 755:229-236.

Gurnsey, M.P., and Johnson, L.A., "Recent improvements in efficiency of flow cytometric sorting of X and Y-chromosome bering sperm of domestic animals: a review", 1998, New Zealand Society of Animal Protection, three pages.

Hall, J. B., et al., "Effect of age and pattern of gain on induction of puberty with a progestin in beef heifers." J. Anim. Sci. 75:1606. 1997

Hamano, K., et al., "Gender Preselection in Cattle with Intracytoplasmically Injected, Flow Cytometrically Sorted Sperm Heads", Biology of Reproduction 60, 1999, pp. 1194-1197.

Harrison, L.A., Squires, E.L. and McKinnon, A.O. 1991. Comparison of hCG, buserelin and luprostiol for induction of ovulation in cycling mares. Eq. Vet. Sci. 3:163-166.

Harte, F. J. "System of production ofbeeffrom once calved heifers." In: J.C. Taylor (Ed.) The early calving ofheifers and it's impact on beef production. 123. 1975

Hawk, H. "Fertilization Rates in superolvulating cows after deposition of semen on the infundidlum near the uterotubal junction or after insemination with high numbers of sperm.", XP-002103478, Biosis, 1988, one page.

Hemlesmeyer, G. N., et al. "Effects of lactation and prenatal androgenization on the perfomlance, carcass coompostion and longissimus muscle sensory characteristics ofheifers in the single-calfheifer system." The Professional Animal Scientist 15:14. 1999

Hennegmeyer, G. N., et al. "Effects of prenatal androgenization and implantation on the performance and carcass composition of lactating heifers in the single-calfheifer system." The Professional Animal Scientist 15:173. 1999

Hilton, G. G., et al., "An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows." I. Anim. Sci. 76:2094. 1998

Ho, L., et al., "Influence of gender, breed and age on maturity characteristics of sheep." J. Anim. Sci. 67:2460-2470. 1989

Hofferer, S., Lecompte, F., Magallon, T., Palmer, E. and Combamous, Y. 1993. Induction of ovulation and superovulation in mares using equine LH and FSH separated by hydrophobic interaction chromatography. J. Reprod. Fert. 98:597-602.

Hohenboken, W. D. "Applications of sexed semen in cattle production." Therio.52:1421. 1999

Holtan, D.W., Douglas, R.H. and Ginther, O.J. 1977. Estrus, ovulation and conception following synchronization with progesterone, prostaglandin F2 ct and human chorionic gonadotropin in pony mares. J. Anim. Sci. 44:431-437.

Householder, D.D., Pickett, B.W., Voss, J.L. and Olar, T.T. 1981. Effect of extender, number of spermatozoa and hCG on equine fertility. J. Equine Vet. Sci. 1:9-13.

Howard, J.G., Roth, T.L., Byers, A.P., Swanson, W.F. and Wildt, D.E. 1997. Sensitivity to exogenous gonadotropins for ovulation and laparoscopic artificial insemination in the theetab and clouded leopard. Biol. Reprod. 56:1059-1068.

Howard, J.G., Bush, M., Morton, C., Morton, F., Wentzel, K. and Wildt, D.E. 1991. Comparative semen cryopreservation in ferrets (Mustela putorious furo) and pregnancies after laparoscopic intrauterine insemination with frozen-thawed spermatozoa. J. Reprod. Fert. 92:109-118.

Hunter, R.H.F. 1980. Transport and storage of spermatozoa in the female reproductive tract. Proc 4th Int. Congr. Artira. Repro. and A.I. 9:227-233.

Hyland, J.H., Ainsworth, C.G.V. and Langsford, D.A. 1988. Gonadotropin-releasing hormone (GnRH) delivered by continuous infusion induces fertile estrus in mares during seasonal acyclicity. Proc. Amer. Assoc. Eq. Prac. 181-190.

Irvine, C.H.G. and Alexander, S.L. 1993. In: Equine Reproduction. Edited by McKirmon and Voss. Lea and Febiger. Philadelphia, London. pp. 37.

Jafar, et al., "Sex Selection in Mammals: A Review", Theriogenology, vol. 46, 1996, pp 191-200.

Jarriage, R. "Age of cows at first calving in France." J.C. Taylor (Ed.) The early calving ofheifers and it's impact on beef production. 10. 1975

Jasko, D.J., Martin, J.M. and Squires, E.L. 1992. Effect of volume and concentration of spermatozoa on embryo recovery in mares. Theriogenology. 37:1233-1239

Johnson, L.A. and Welch, G.R., "Sex Preselection: High-speed flow cytometric sorting of X and Y sperm for maximum efficiency", Theriogenology, Vol. 52, (1999), pp. 1323-1341

Johnson, L.A., et al, "Sex Preselection in Swine: Flow Cytometric Sorting of X- and Y- Chromosome

Bearing Sperm to Produce Offspring", Boar Semen Preservation IV, 2000, pp. 107-114.

Johnson, L.A. 1988. Flow cytometric determination of spermatozoa sex ratio in semen purportedly enriched for X or Y bearing spermatozoa. Theriogenology. 29:265. abstr.

Johnson, A.L. and Becker, S.E. 1988. Use of gonadotropin-releasing hormone (GnRH) treatment to induce multiple ovulations in the anestrous mare. Eq. Vet. Sci. 8:130-134.

Johnson, "Gender preselection in Mammals: An overview", Dtsch. tierarztl. Wschr, Vol. 103, Aug./Sep. 1996, pp 288-291.

Johnson, L.A., et al., 'Sex Preselection in Rabbits: Live Births from X and Y Sperm Separated by DNA and Cell Sorting", Execptional Paper-Rapid Publication, XP-002103476, Biology of Reproduction 41, 199-203, 1989, pp 199-203.

Johnson, L.A., Welch, G.R., Rens, W. and Dobrinsky, J.R. 1998. Enhanced flow cytometric sorting of manunalian X and Ysperm: high speed sorting and orienting no77.1e for artificial insemination. Theriogenology. 49(1):361. abstr.

Johnson, L.A. 1992. Gender preselection in domestic animals using flow cytometrically sorted sperm. J Anim. Sci. Suppl 1.70:8-18.

Johnson, L.A. 1994. Isolation of X- and Y-bearing spermatozoa for sex preselection. *In:* Oxford Reviews of Reproductive Biology. Ed. HH Charlton. Oxford University Press. 303-326.

Johnson, L.A., and Pinkel, D., 'Modification of a Laser-Based flow Cytometer for High-Resolution DNA Analysis of Mammalian Spermatozoa", Cytometry 7, 1986, pp 268 - 273.

Johnson, A.L. 1986. Pulsatile release of gonadotropin releasing hormone advances ovulation in cycling mares. B iol. Reprod. 35:1123; 1130.

Johnson, L.A., et al., 1994. Improved flow sorting resolution of X- and Y- chromosome bering viable sperm separation using dual staining and dead cell gating. Cytometry 17 (suppl 7):83.

Johnson, L.A., Flook, J.P., Look, M.V. and Pinkel, D. 1987b. Flow sorting of X and Y chromosome bearing spermatozoa into two populations. Gam. Res. 16:203-212.

Johnson, L..A., "Advances in Gender Preselection in Swine" Journal of Reproduction and Fertility Supplement, Vol. 52, pp. 255-266 (1997)

Johnson, L..A., "Sex Preselection in Swine: Altered Sex Ratios in Offspring Following Surgical Insemination of Flow Sorted X- and Y- Bearing Sperm", Reproduction in Domestic Animals, Vol. 26, pp. 309-314 (1991)

Johnson L.A., et al., 1987. Flow cytometry of X- and Y- chromosome bearing sperm for DNA using an improved preparation method and staining with Hoechst 333-42. Garnete Research 17: 203-212

Johnson, L.A. and Schulman, J.D. 1994. The safety of sperm selection by flow cytometry. Ham. Reprod. 9(5):758.

Johnson, L.A. 1995. Sex preselection by flow cytometric separation of X and Y chromosome bearing spermatozoa based on DNA difference: a review. Reprod. Fert. Dev. 7:893-903.

Joseph, R. L. and J. P. Crowley. "Meat quality of once-calved heifers." Irish J. of Agric. Research 10:281.

Joseph, R. L. "Carcass composition and meat quality in once calved heifers." In: J.C. Taylor (Ed.) The early calving ofheifers and it's impact on beef production. 143. 1975

Kachel, V., et al., "Uniform Lateral Orientation, Cused by Flow Forces, of Flat Particles in Flow-Through Systems", The Journal of Histochemistry and Cytochemistry, 1997, Vol. 25, No. 7, pp 774 -780.

Kanayama, K., Sankai, T., Nariaik, K., Endo, T. and Sakuma, Y. 1992b. Pregnancy by means of tubal insemination and subsequent spontaneous pregnancy in rabbits. J. Int. Med. Res. 20:401-405.

Karabinus, et al., "Effects of Egg Yolk-Citrate and Milk Extenders on Chromatin Structured Viability of Cryopreserved Bull Sperm", Journal of Dairy Science, Vol. 74, No. 11, 1999, pp 3836-3848.

Keeling, P. C. B. M. S. T. G. D. I. a. P. W. J., "A modelling study of once-bred heifer beef production." Proceedings of the New Zealand Society of Animal Production. 51. 1991

Kilicarslan, M.R., Horoz, H., Senunver, S.C., Konuk, S.C., Tek, C. and Cariogiu, B. 1996. Effect of GrnRH and hCG on ovulation and pregnancy in mares. Vet. Rec. 139:119-120.

Kinder, J. E., et al., 1987. Endocrine regulation of puberty in cows and ewes." J. of Repro. and Fertility, Suppl. 34:167. 1987

Kinder, J. E., et al. "Endocrine basis for puberty in heifers and ewes." J. Repro. and Fertility 393. 1995

Klindt, J. and J. D. Crouse. "Effect of ovariectomy and ovariectomy with ovarian auto transplantation on feedlot performance and carcass characteristics ofheifers." J. Anim. Sci. 68:3481. 1990

Klosterman, E. W. and C. F. Parker. "Effect of size, beed and sex upon feed efficiency in beef cattle."
North Central Regional Research Publication 235, Ohio Agric. Research and Development Center 1090:3.
1976

Kniffen, D. M., Wagner, W.R., and Lewis. P.E. "Effects oflong-tenn estrogen implants in beef heifers." I. Anim. Sci. 77:2886. 1999

Koch, R. M., et al., "Characterization of biological types of cattle -Cycle-II .3." Carcass composition, quality and palatability. I. Anim. Sci. 49:448. 1919

Lapin, D.R. and Ginther, O.J. 1977. Induction of ovulation and multiple ovulations in seasonally anovulatory and ovulatory mares with an equine pituitary extract. J. Anim. Sci. 44:834-842.

Laster, D. B., "Factors affecting dystocia and effects of dystocia on subsequent reproduction in beef-cattle." J. Anim. Sci. 36:695. 1973

Lawrenz, R. 1985. Preliminary results of non-surgical intrauterine insemination of sheep with thawed frozen semen. J S Afr. Vet. Assoc. 56(2):61-63.

Levinson, G., Keyvanfar, K., Wu, J.C., Fugger, E.F., Fields, R.A., Harton, G.L., Palmer, F.T., Sisson, M.E., Starr, K.M., Dennison-Lagos, L., Calvo, L., Sherins, R.J., Bick, D., Schulman, J.D. and Black, S.H. 1995. DNA-based X-enriched sperm separation as an adjunct to preimplantation genetic testing for the preparation of X-linked disease. Mol. Human Reprod. 10:979-982.

Lindsey, A., et al., "Hysteroscopic Insemination of Mares with Nonfrozen Low-dose Unsexed or Sexsorted Spermatozoa", currently unpublished, pp. 1-15.

Linge, F. 1972. Faltforsok med djupfrost sperma (field trials with frozen sperm). Farskotsel. 52:12-13.

Lonergan, P., et al., "Effect of Time Interval from Insemination to First Cleavage on the Development of Bovine Embryos In Vitro and In Vivo", Theriogenology, 1999, p. 326

Long, C.R., Rath, D., Welch, G.R., Schreier, L.L., Dobrinsky, J.R. and Johnson, L.A. 1998. □In vitro production of porcine embryos from semen sorted for sex with a high speed cell sorter: comparison of two fertilization media. □, Theriogenology. 49(1):363. abstr.

Long, C.R., Rath, D., Welch, G.R., Schreier, L.L., Dobrinsky, J.R. and Johnson, L.A. 1998. Theriogenology. 49(1):363. abstr.

Loy, R.G. and Hughes, J.P. 1965. The effects of human chorionic gonadotropin on ovulation, length of estrus, and fertility in the mare. Cornell Vet. 56:41-50.

Lu, K.H., et al., 'In Vitro Fertilization with Flow-Cytometrically-Sorted Bovine Sperm', Theriogenology 52, 1999, pp. 1393-1405.

Lynch, I. M., et al., "Influence of timing of gain on growth and reproductive performance ofbeefreplacement heifers." I. Anim. Sci. 75:1715. 1997

Macmillan, K.L. and A.M. Day, "Prostaglandin F2a - A Fertility Drug In Dairy Cattle?", Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand, Theriogenology, September 1982, Vol. 18 No. 3, pages 245-253

Martin, A. H., et al., "Characteristics of youthful beef carcasses in relation to weight, age and sex .3. meat quality attributes." Canadian I. Anim. Sci. 51:305. 1971

Martin, L. C., J. S. Brinks, R. M. Bourdon, and L. V. Cundiff. "Genetic-effects on beef heifer puberty and subsequent reproduction." J. Anim. Sci. 70:4006. 1992

Matsuda, Y. and Tobari, I. 1988. Chromosomal analysis in mouse eggs fertilized in vitro with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulfonate (MMS and EMS). Mutat. Res.

198:131-144.

Matulis, R. J., F. K. Mckeith, D. B. Faulkner, L. L. Berger, and P. George. "Growth and carcass characteristics of cull cows after different times-on-feed." J. Anim. Sci. 65:669. 1987

Mauleon, P. "Recent research related to the physiology of puberty." Commission of the European Communities. The early calving of heifers and it's impact on beef production. 1975

Maxwell, W.M.C., Evans, G., Rhodes, S.L., Hillard, M.A. and Bindon, B.M. 1993. Fertility of Superovulated Ewes after Intrauterine or Oviductal Insemination with Low Numbers of Fresh or Frozen-Thawed Spermatozoa. Reprod. Fertil. Dev. 5:57-63.

Maxwell, W. and Johnson, L., "Chlortetracycline Analysis of Boar Spermatozoa after Incubation, Flow Cytometric Sorting, Cooling, or Cryopreservation", Molecular Reproduction and Development 46, 1997, pp. 408-418.

Mccomlick, R. J. "The flexibility of the collagen compartment of muscle." Meat Sci. 36:79. 1994

McCue, P.M., Fleury, J.J., Denniston, D.J., Graham, J.K. and Squires, E.L. 1997. Oviductal insemination in the mare. 7th Int Symp. Eq. Reprod. 133. abstr.

McCue, P.M. 1996. Superovulation. Vet. Clin. N. Amer. Eq. Prac. 12:1-11.

McDonald, L.E. 1988. Hormones of the pituitary gland. *In:* Veterinary Pharmacology and Therapeutics. 6 th ed. Edited by N.H. Booth and L.E. McDonald. Ames, Iowa State Univ. Press. pp. 590.

McKenna, T., Lenz, R.W., Fenton, S.E. and Ax, R.L. 1990. Nonreturn rates of dairy cattle following uterine body or comual insemination. J. Dairy Sci. 73:1179-1783.

McKinnon, A. and Voss, J., "Equine Reproduction", Lea & Febiger, Philadelphia, 1993, pp 291, 299 - 302, 345 - 348, 739 - 797.

McKinnon, A. et al, 1993. Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. Eq. Vet. J. 25:321-323.

McKinnon, A.O. et al, 1996. Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in the transitional mare. Eq. Vet. J. 29:153-155.

McNutt, M. and Johnson, L., "Flow Cytometric Sorting of Sperm: Influence on Fertilization and Embryo/Fetal Development in the Rabbit", Molecular Reproduction and Development 43, 1996, 261-267.

Meilgaard, M., G. V. Civille, and B. T. Carr. "Sensor Evaluation Techniques." CRC Press Inc., Boca Raton, FL. 1991

Meinert, C., et al., "Advancing the time of ovulation in the mare with a short-term inplant releasing the GnRH analogue deslorelin", Equine Veterinary Journal, 25, 1993, pp 65 - 68.

Merton, J., et al., "Effect of Flow Cytometrically Sorted Frozen/Thawed Semen on Success Rate of In Vitro Bovine Embryo Production", Theriogenology 47, 1997, pp. 295.

Meyers, P.J., Bowman, T., Blodgett, G., Conboy, H.S., Gimenez, T., Reid, M.P., Taylor, B.C., Thayer, J., Jochle, W. and Trigg, T.E. 1997. Use of the GnRH analogue, deslorelin acetate, in a slow release implant to accelerate ovulation in oestrous mares. Vet. Rec. 140:249-252.

Michaels, Charles, "Beef A.I. Facilities that work", Proc. Fifth N.A.A.B Tech. Conf. A.I. Reprod. Columbia, MO. pp. 20-22.

Michel, T.H., Rossdale, P.D. and Cash, R.S.G. 1986. Efficacy of human chorionic gonadotrophin and gonadatrophin releasing hormone for hastening ovulation in Thoroughbred mares. Eq. Vet. J. 6:438-442.

Miller, S.J. 1986. Artificial Breeding Techniques in Sheep. In Morrow, D.A. (ed): Current Therapy in Theriogenology 2. Philadelphia, WB Saunders.

Mirskaja, L.M. and Petrapavlovskii, V.V. 1937. The reproduction of normal duration of heat in the mare by the administration of Prolan. Probl. Zivotn. Anim. Breed. Abstr. 5:387.

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. "Energetics ofbodytissue mobilization." J. of Dairy Sci. 54:548.

Molinia, F.C., Gibson, R.J., Brown, A.M., Glazier, A.M. and Rodger, J.C. 1998. Successful fertilization after superovulation and laparoscopic intrauterine insemination of the brushtail possum, *Trichosurus*

vulpecula, and tammar wallaby, Macropus eugenii. J.Reprod. Fert. 112:9-17.

Moms, s. T., et al., "Biological efficiency: How relevent is this concept to beef cows in a mixed livestock seasonal pasture supply context?" Proceedings of the New Zealand Society of Animal Production 54:333. 1994

Monensin." J. Anim. Sci. 55:357-362. 1982

Moran, C., J. F. Quirke, and J. F. Roche. "Puberty in heifers -a review." Animal Reproduction Sci. 18:167. 1989

Morcom, C.B. and Dukelow, W.R. 1980. A research technique for the oviductal insemination of pigs using laparoscopy. Lab. Anim. Sci. 1030-I031.

Morgan, J. B., et al., "National beef tenderness survey." J. Anim. Sci.69:3274. 1991

Morris, L.H., et al., "Hysteroscopic insemination of small numbers of spermatozoa at the uterotubal junction of preovulatory mares", Journal of Reproduction and Fertility, Vol. 118, pp. 95-100 (2000)

Moseley, W. M., et al., 1982. "Relationship of Growth and Puberty in Beef Heifers Fed

Mount, D. E. "Fibrous and non-fibrous carbohydrate supplementation to ruminants grazing forage from small grain crops." M.S. Thesis. Colorado State University. 2000

Muller, W. and Gautier, F. 1975. Interactions of heteroaromatic compounds with nucleic acids. Euro. J Biochem. 54:358.

Munne, S. 1994. Flow cytometry separation of X and Y spermatozoa could be detrimental to human embryos. Hum. Reprod. 9(5):758

Myers, S. E., et al., "Comparison of three weaning ages on cow-calfperformance and steer carcass traits." J. Anim. Sci. 77:323. 1999

Myers, S. E., "Performance and carcass traits of early-weaned steers receiving either a pasture growing period or a finishing diet at weaning." J. Anim. Sci. 77:311. 1999

Myers, S. E., et al., "Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers." J. Anim. Sci. 77:300. 1999

Nix, I. P., I. C. Spitzer, and P. I. Chenoweth. "Serum testosterone concentration, efficiency of estrus detection and libido expression in androgenized beef cows." Therio. 49: 1195. 1998

Nowshari, et al., "Superovulation of Goats with Purified pFSH Supplemented with Defined Amounts of pLH", Theriogenology, Vol 43, 1995, pp 797-802.

Nowshari, et al., Theriogenology, Vol 43, 1995, pp 797-802.

NRC. Nutrient requirements for beef cattle. National Academy of Sci. National Research Council, Washington, DC. 1996

Olson, S.E. and Seidel, G.E. Jr., "Reduced Oxygen Tension and EDTA improve Bovine Zygote Development in a Chemically Defined Medium", Journal of Animal Science 78, 2000, pp. 152-157.

Owen, J. B. "The maiden female-a means of increasing meat production." Proc. Symp. on the use of once bred heifers and gilts. 1973

Pace, M.M. and Sullivan, J.J. 1975. Effect of timing of insemination, numbers of spermatozoa and extender components on pregnancy rates in mares inseminated with frozen stallion semen. J Reprod. Fert. Suppl. 23:115-121.

Parent US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.

Parrish, J.J. "Capacitation of Bovine Sperm by Heparin", Department of Meat and Animal Science, University of Wisconsin, Madison, WI 53706, Biology Of Reproduction 38, 1988, pp 1171-1180.

Parrish, J., et al., "Capacitation of Bovine Sperm by Heparin", Technology of Reproduction 38, 1988, pp. 1171-1180.

PCT application PCT/US98/27909, filed 31 December 1998, entitled "Commercially Practical Sex-Specific Insemination of Mammals".

PCT application PCT/US99/17165, filed 28 July 1999, entitled "Equine System for Non-Surgical Artificial Insemination".

Peippo, J., et al., "Sex diagnosis of equine preimplantation embryos using the polymerase chain reaction", Theriogenology, Vol. 44 619-627 (1995)

Perry, E.J. 1968. Historical Background In: The Artificial Insemination of Farm Animals. 4th ed. Edited by E.J. Perry. New Brunswick, Rutgers University Press, pp. 3-12.

Petersen, G.A., et al, "Cow and Calf Performance and Economic Considerations of Early Weaning of Fall-Born Beef Claves", J. Anim. Sci., 1987, 64:15, pp 15-22.

Peterson, G. A., et al., "Cow and calfperfomlance and economic-considerations of early weaning offall-bom beef-calves." J. Anim. Sci. 64:15. 1987

Petit, M. "Early Calving in Suckling Herds." In: (Ed.) J.C. Taylor. The early calving of heifers and it's impact on beef production. 157. 1975

Pickett, B.W., Burwash, L.D., Voss, J.L. and Back, D.G. 1975b. Effect of seminal extenders on equine fertility. J. Anim. Sci. 40:1136-1143.

Pickett GW, et al., "Management of the mare for maximum reproductive efficiency", Bulletin No. 6 Colorado State University, Ft. Collins CO. (1989)

Pickett, B.W. and Back, D.G. 1973. Procedures for preparation, collection, evaluation and insemination of stallion semen. C.S.U. Exp. Sta. Artira. Reprod. Lab. Gen. Series Bull. 935.

Pickett, B.W, et al., 1976. Factors influencing the fertility of stallion spermatozoa in an A.I. program. Proc. 8th Internat. Congr. Anim. Reprod. A.I. Krakow, Poland. 4: 1049 - 1052.

Pickett GW, et al., "Management of the mare for maximum reproductive efficiency" Bulletin No. 6 Colorado State University, Ft. Collins CO. (1989)

Pickett, B.W., and Shiner, K.A., "Recent developments in artificial insemination in horses", Livestock Production Science, 40, 1994, pp 31 - 36.

Pinkel, D., Gledhill, B.L., Van Dilla, M.A., Stephenson, D. and Watchmaker, G. 1982b. High resolution DNA measurements of mammalian spermatozoa. Cytometry. 3:1-9. (1982b)

Pinkel, D., et al, "Flow Cytometric Determination of the Proportions of X- and Y- Chromosome-Bearing Sperm in Samples of Purportedly Separated Bull Sperm", Journal of Animal Science, Vol. 60, No. 5, 1985, pp 1303 - 1307.

Polge, E. J., "Historical Perspective of AI: Commercial Methods of Producing Sex Specific Semen, IVF Procedures", Proceedings of the 16th Technical Conference on Artificial Insemination & Reproduction, Cambridge, England, 1996, pp. 7-11.

Purvis, H. T. and J. C. Whittier. "Effects ofionophore feeding and anthelmintic administration on age and weight at puberty in spring-bom beef heifers." J. Anim. Sci. 74:736-744. 1996

Randel, R. D. "Nutrition and postpartum rebreeding in cattle." J. Anim. Sci. 68:853. 1990

Rath, D., et al., "Low Dose Insemination Technique in the Pig", Boar Semen Preservation IV, 2000, pp. 115-118.

Rath, D., et al., "Production of Piglets Preselected for Sex Following in Vitro Fertilization with X and Y Chromosome-Bearing Spermatozoa Sorted by Flow Cytometry", Theriogenology, 47, 1997, pp 795 - 800.

Reiling, B. A., "Effect of prenatal androgenization on perfonnance, lactation, carcass, and sensory traits ofheifers in a single-calfheifer system." J. Anim. Sci. 73:986. 1995

Reiling, B.A., et al., "Effect of Prenatal Androgenization on Preformance, Location, and Carcass and Sensory Traits on Heifers in Single Calf Heifer System", J. Anim. Sci., 1995, 73: 986, pp 986-992.

Reiling, B. A., "Effects of prenatal androgenization, melengestrol acetate, and synovex-h(r) on feedlot performance, carcass, and sensory traits of once-calved heifers." J. Anim. Sci. 74:2043. 1996

Reiling, B. A., J. K. Drackley, L. R. Grum, and L. L. Berger. "Effects of prenatal androgenization and lactation on adipose tissue metabolism in finishing single-calfheifers." J. Anim. Sci. 75:1504. 1997

Rens, W., et al., "A Novel Nozzle for More Efficient Sperm Orientation to Improve Sorting Efficiency of

X and Y Chromosome-Bearing Sperm", Technical Notes, Cytometry 33, 1998, pp 476-481.

Rens, W., et al., "Improved Flow Cytometric Sorting of X- and Y- Chromosome Bearing Sperm: Substantial Increase in Yield of Sexed Semen", Molecular Reproduction and Development, 1999, pp 50-56.

Rieger, D., et al, "The Relationship Between the Time of First Cleavage of Fertilized Cattle Oocytes and Their Development to the Blastocyst Stage", Theriogenology, 1999, pp. 190.

Ritar, A. and Ball, A. 1991. Fertility of young cashmere goats after laparoscopic insemination. J. Agr. Sci. 117:271-273.

Roberts, J.R. 1971. In: Veterinary Obstetrics and Genital Diseases. Ithaca, New York. pp. 740-749.

Romita, A. "Some considerations on the beef situation in Italy." (Ed.) J.C. Taylor. The early calving of heifers and it's impact on beef production. 23. 1975

Roser, JF., Evans, J.W., Kiefer, DP., Neeley, D.P. and Pacheco, C.A. 1980. Reproductive efficiency in mares with anti-hCG antibodies. Proc 9th Int. Congr. Artira. Repro. and A.I. 4:627. abstr.

Roth, T.L., Wolfe, B.A., Long, J.A., Howard, J. and Wildt, D.E. 1997. Effects of equine chorionic gonadotropin, human chorionic gonadotropin, and laparoscopic artificial insemination on embryo, endocrine, and luteal characteristics in the domestic cat. Bio Reprod. 57:165-171.

Roux, M., J. H. Teissier, J. Bonnemaire, and R. Dumont. "Early calving heifers versus maiden heifers for beef-production from dairy herds. 1." The effects of genotype (Friesian and Charolais x Friesian) and 2 feeding levels in the rearing period on growth and carcass quality. Livestock Prod. Sci.16:1. 1987

Rowley, H-S., Squires, E.L. and Pickett, B.W. 1990. Effect of insemination volume on embryo recover}' in mares. J. Equine Vet. Sci. 10:298-300.

Roy, J. H. B. "Rearing dairy-herd replacements." J. of the Soc. of Dairy Technology 31:73-79. 1978

Rutter, L. M., et al., "Effect of abomasal infusion of propionate on the GnRH-induced luteinizing-hormone release in prepuberal heifers." J. Anim. Sci. 56:1167. 1983

Salamon, S. 1976. Artificial Insemination of Sheep. Chippendale, New South Whales. Publicity Press. p.83-84.

Salisbury, G.W. and VanDemark, N.L. 1961. Physiology of Reproduction and Artificial Insemination of Cattle. San Francisco: Freeman and Company.

SAS, SAS/STAT; User's Guide (Release 6.03), SAS Inst. Inc., Cary, NC., 1988. 3 pages

SAS. "The SAS System for Windows." Ver 7.0. ReI 6.12. SAS Inst.lnc., Cary, NC. 2000

Schenk, J. L., T. K. Suh, D. G. Cran, and G. E. Seidel. "Cryopreservation of flow-sorted bovine spennatozoa." Therio. 52:1375. 1999

Schenk, J.L., "Cryopreservation of flow-sorted bovine spermatozoa", Theriogenology, Vol. 52, 1375-1391 (1999)

Schenk, J.L. and Seidel, Jr., G.E., "Imminent Commercialization of Sexed Bovine", Proceedings, The Range Beef Cow Symposium XVL, 1999, pp 89-96.

Schillo, K. K., J. B. Hall, and S. M. Hileman. "Effects of nutrition and season on the onset of puberty in the beef heifer." J. Anim. Sci. 70:3994. 1992

Schmid R.L., et al, "Fertilization with Sexed Equine Spermatozoa Using Intracytoplasmic Sperm Injection and Oviductal Insemination", 7th International Symposium On Equine Reproduction, pp. 139 (Abstract) (1998)

Schnell, T. D., K. E. Belk, J. D. Tatum, R. K. Miller, and G. C. Smith. "Performance, carcass, and palatability traits for cull cows fed high-energy concentrate diets for 0, 14,28,42, or 56 days." J. Anim. Sci. 75:1195.

Schoonmaker, J. P., et al., "Effects of age at weaning and implant strategy on growth of steer calves." J. Anim. Sci. (SuppI2) 76:71 (Abstr.). 1998

Seidel, G. E., "Insemination of heifers with sexed sperm." Therio. 52:1407. 1999

- Seidel, G. E. and L. A. Johnson. "Sexing mammalian spenn -overview." Therio. 52: 1267. 1999
- Seidel, Jr., G.E. et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa", Colorado State University (1996)
- Seidel, G.E. Jr., Cran, D.G., Herickoff, L.A., Schenk, J.L., Doyle, S.P. and Green, R.D. 1999. Insemination of heifers with sexed frozen or sexed liquid semen. Theriogenology. 51. (in press). abstr.(1999)
- Seidel, G. Jr., "Use of Sexed Bovine Sperm for In Vitro Fertilization and Superovulation, Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, Proceedings of the 2000 CETA/ACTE Convention, Charlottetown, Prince Edward Island, August 2000, pp. 22-24.
- Seidel, Jr., G. E., et al, "Insemination of Holstein Heifers With Very Low Numbers Of Unfrozen Spermatozoa", Colorado State University, Atlantic Breeders Cooperative, (1995)
- Seidel, Jr., G. E., "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, (1996)
- Seidel, G.E. Jr, et al., "Insemination of Heifers with Sexed Sperm", Theriogenology, Vol. 52, pp. 1407-1421 (1999)
- Seidel, G.E. Jr, et al., "Artificial Insemination of Heifers with Cooled, Unfrozen Sexed Semen", Theriogenology, Vol. 49 pp. 365 (Abstract) (1998)
- Seidel, G.E. Jr., "Uterine Horn Insemination of Heifers With Very Low Numbers of Nonfrozen and Sexed Spermatozoa", Atlantic Breeders Cooperative, Theriogenology 48: pp. 1255-1264, (1997)
- Sell, R. S., D. L. Watt, R. D. Little, and T. A. Petry. "Single-calfheifer profitability compared to other north dakota beef production systems." Department of Ag. Eco., North Dakota State University, Ag. Econ. Rpt. 20.
- Senger, P.L., Becker, W.C., Davidge, S.T., Hillers, J.K. and Reeves, J.J. 1988. Influence of comual insemination on conception rates in dairy cattle. J Anim. Sci. 66:3010-3016.
- Shackelford, S. D., M. Koohmaraie, and T. L. Wheeler. "Effects of slaughter age on meat tenderness and usda carcass maturity scores of beef females." I. Anim. Sci. 73:3304. 1995
- Shelton, J.N. and Moore, N.W. 1967. The response of the ewe tot pregnant mare gonadotropin and to horse anterior pituitary extract. J. Reprod. Fert. 14:175 177.
- Shilova, A.V., Platov, E.M. and Lebedev, S.G. 1976. The use of human chorionic gonadothrophin for ovulation date regulation in mares. VIIIth Int. Congr. On Anim. Repro. and A.I. 204-208.
- Shorthose, W. R. and P. V. Harris. "Effect of animal age on the tenderness of selected beef muscles." I. Food Sci. 55:1-. 1990
- Siedel, G.E., "Status of Sexing Semen for Beef Cattle", Texas A&M University 45th Annual Beef Cattle Short Course and Trade Show Proceedings, August 9-11, 1999; pp.III 24-III 27
- Silbennann, M., "Honnones and Cartilage: Cartilage: development, differentiation, and growth." pp. 327-368. Academic Press, Inc. 1983
- Simon, M., "The effect of management option on the perfonnance of pregnant feedlot heifers." M.S. Thesis. Kansas State University. 1983
- Smith, G. C., B. W. Berry, J. W. Savell, and H. R. Cross. "USDA maturity indexes and palatability ofbeefrib steaks." J. of Food Quality 11:1. 1988
- Smith, G. C., et al., "Relationship ofusda maturity groups to palatability of cooked beef." J. of Food Sci. 47:1100. 1982
- Squires, E.L., "Early Embryonic Loss" in Equine Diagnostic Ultrasonography, 1st Ed. pp 157-163 Eds Rantanen & McKinnon. Williams and Wilkins, Baltimore, Maryland (1998)
- Squires, E.L, Moran, D.M., Farlin, ME., Jasko, D.J., Keefe, T.J., Meyers, S.A., Figueiredo, E., McCue, P.M. and Jochle, W. 1994. Effect of dose of GnRH analogue on ovulation in mares. Theriogenology. 41:757-769.
- Squires, E., "Simultaneous Analysis of Multiple Sperm Attributes by Flow Cytometry", Diagnostic Techniques and Assisted Reproductive Technology, The Veterinary Clinics of North America, Equine

Practice, Vol. 12, No. 1, April 1996, pp127 - 130.

Squires, E.L.., et al, "Cooled and frozen stallion semen", Bulletin No. 9, Colorado State University, Ft. Collins, CO. (1999)

Stellflug, J. N., D. K. Ran, R. D. Randel, and Eo L. Moody. "Plasma estrogens in peri-parturient cow." Therio 10:269. 1978

Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. Lefever. "Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beefheifers." J. Anim. Sci. 74:729. 1996

Story, C. E., R. J. Rasby, R. T. Clark, and C. T. Milton. "Age of calf at weaning of spring-calving beef cows and the effect on cow and calf perfomlance and production economics." J. Anim. Sci. 78:1403. 2000

Sullivan, J.J., Parker, W.G. and Larson, LL. 1973. Duration of estrus and ovulation time in nonlactating mares given human chorionic gonadotropin during three successive estrous periods. J.A.V.M.A. 162:895-898.

Swanson, E. W. "Future research on problems of increasing meat production by early calving." Comm. Eur. Commun., Eur. 5545.1975. The Early Calving ofHeifers and its Impact on Beef Production.

Taljaard, T.L., Terblanche, S.J., Bertschinger, H.J. and Van Vuuren, L.J. 1991. The effect of the laparoscopic insemination technique on the oestrus cycle of the ewe. J. S Afr. Vet. Assoc. 62(2):60-61.

Tatum, J. D., G. C. Smith, B. W. Berry, C. E. Murphey, F. L. Williams, and Z. L. Carpenter. "Carcass characteristics, time on feed and cooked beef palatability attributes." J. Anim. Sci. 50:833. 1980

Taylor, S. C. S., A. J. Moore, R. B. Thiessen, and C. M. Bailey. "Efficiency of food utilization in traditional and sex-controlled systems of beef-production." Animal Production 40:401. 1985

Taylor, C.S., Moore, A.J. Thiessen, R.B. and Bailey, C.M., AFRC Animal Breeding Research Organisation, West Mains Road, Edinburg EH9 3JQ, "Efficiency of Food Utilization in Traditional and Sex-Controlled Systems of Beef Production", pp 401-440.

Tervit, H.R., et al., "Successful Culture In Vitro of Sheep and Cattle Ova", Agricultural Research Council, Unit of Reproduction Physiology and Biochemistry, University of Cambridge, 1972, p. 493-497.

Unruh, J. A. "Effects of endogenous and exogenous growth-promoting compounds on carcass composition, meat quality and meat nutritional-valu-." J. Anim. Sci. 62:1441. 1986

US Application 60/211093, entitled □Integrated System for Herd Management Using Sexed Semen□, filed June 12, 2000.

US Application, 09/511,959 entitled □Methods For Improving Sheath Fluids and Collection Systems For Sex-Specific Cytometer Sorting of Sperm□, filed February 23, 2001.

US Application 09/015, 454, entitled □System for Improving Yield of Sexed Embryos in Mammals□, filed on January 29, 1998, 59 total pages which includes drawings.

US Application, 09/511,959 entitled □Methods For Improving Sheath Fluids and Collection Systems For Sex-Specific Cytometer Sorting of Sperm□, filed February 23, 2001.

US Application 60/211093, entitled □Integrated System for Herd Management Using Sexed Semen□, filed June 12, 2000.

US Application, 09/454,488, entitled □Improved Flow Cytometer Nozzle and Flow Cytometer Sample Handling Methods□, filed December 3, 1999.

Application, 60/224,050, entitled □ Integrated System for Herd Management With Terminal-Cross Program
Using Sexed Semen□, filed August 9, 2000.

US Application, 60/238,294, entitled ∃Hysteroscopic Insemination of Mares ☐ filed October 5, 2000.

US Application Serial Number 60/203,089, entitled Detector System for Resolving Small Differences in Photo-generated Signal , filed May 9, 2000.

US Application Serial Number 60/113,143, entitled □Equine Insemination System □, December 18, 1998.

US Application 09/001,394, entitled □Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm□, filed on December 31, 1997, 87 total pages which includes four drawings.

US Application Serial Number 60/094,720, entitled □System for Low Dose Insemination of Equines□, filed July 30, 1998.

US Application Serial Number 60/224,050., entitled □Integrated System for Herd Management With Terminal-Cross Program Using Sexed Semen□, filed August 9, 2000.

US Application entitled □System For Separating Frozen-Thawed Sperm Cells Into X-Chromosome And Y-Chromosome Bearing Populations□, filed November 28, 2000.

US Application, 09/448,643, entiled □Multiple Sexed Embryo Production System for Mammals□, filed November 24, 1999.

US Application, 09/454,488, entitled □Improved Flow Cytometer Nozzle and Flow Cytometer Sample Handling Methods□, filed December 3, 1999.

USDA "Official United States standards for grades of carcass beef." Agric, Marketing Serv., USDA .Washington, DC. 1997

Vazquez, J. et al., □Nonsurgical Uterotubal Insemination in the Mare□, Proceedings of the 44th Annual Convention of the American Association of Equine Practitioners, Baltimore, Maryland, December 6-9, 1998, Vol. 44, pp 68-69

Vazquez, J., et al., □Development of a Non-surgical Deep Intra Uterine Insemination Technique□, IV International Conference on Boar Semen Preservation, Maryland, August, 1999, p 35 and photo of display board.

Vazquez, J., et al., □A.I. in Swine; New Strategy for Deep Insemination with Low Number of Spermatozoa Using a Non-surgical Methodology□, 14th International Congress on Animal Reproduction, Vol. 2, Stockhlom, July, 2000, p. 289.

Vazquez, J., et al., □Successful Low-Dose Insemination by a Fiberoptic Endoscope Technique in the Sow □, Proceedings Annual Conference of the International Embryo Transfer Society, Netherlands, Theriogenology, Vol. 53, January, 2000, pp. 201.

Vazquez, J., et al.,□Hypoosmotic Swelling Test as Predictor of the Membrane Integrity in Boar Spermatozoa□□, Boar Semen Preservation IV, IVth International Conference on Boar Semen Preservation, Maryland, pp. 263.

Vidament, M., Dupere, A.M., Julienne, P., Evain, A., Noue, P. and Palmer, E. 1997. Equine frozen semen freezeability and fertility field results. Theriogenology. 48:907.

Vidament, M., Dupere, A.M., Julienne, P., Evain, A., Noue, P. and Palmer, E. 1997. Equine frozen semen freezeability and fertility field results. Theriogenology. 48:907.

Vincent, B. C., S. D. M. Jones, L. E. Jeremiah, M. A. Price, and J. A. Newman. "Carcass characteristics and meat quality of once-calved heifers." Canadian J. Anim. Sci. 71:311. 1991

Voss, J.L. and Pickett, B.W. 1976. Reproductive management of the broodmare. C.S.U. Exp. Sta. Anim. Reprod. Lab. Gen. Series. Bull. 1-12

Voss, J.L., Squires, E.L., Pickett, B.W., Shideler, R.K. and Eikenberry, D.J. 1982. Effect of number and frequency of inseminations on fertility in mares. J. Reprod. Fertil. Suppl. 32:53-57.

Voss, J.L., Pickett, B.W., Burwash, L.D. and Daniels, W.H. 1974. Effect of human chorionic gonadotropin on duration of estrous cycle and fertility of normally cycling, nonlactating mares. J.A.V.M.A. 165:704-706.

Waggoner, A. W., M. E. Dikeman, I. R. Brethour, and K. E. Kemp. "Performance, carcass, cartilage calcium, sensory and collagen traits of longissimus muscles of open versus 30-month-old heifers that produced one calf." I. Anim. Sci. 68:2380. 1990

Welch G.R., et al., 1994. Fluidic and optical modifications to a FACS IV for flow sorting of X- and Y-chromosome bearing sperm based on DNA. Cytometry 17 (suppl. 7): 74.

Welch, G., et al., □Flow Cytometric Sperm Sorting and PCR to Confirm Separation of X- and Y-Chromosome Bearing Bovine Sperm□, Animal Biotechnology, 6 (2), 131-139, 1995, pp 131 - 139.

Wheeler, T. L., L. v. Cundiff, and R. M. Koch. "Effect of marbling degree on beef palatability in Bos-Taurus and Bos-Indicus cattle." J. Anim. Sci. 72:3145. 1994

Wickersham, E. W. and L. H. Schultz. "Infilience of age at first breeding on growth, reproduction, and

production of well-fed holstein heifers." J. Dairy Sci. 46:544. 1963

Wilson, M.S. 1993. Non-surgical intrauterine artificial insemination in bitches using frozen semen. J.Reprod. Fert Suppl. 47:307-311.

Wilson, C.G., Downie, C.R., Hughes, J.P. and Roser, J.F. 1990. Effects of repeated hCG injections on reproductive efficiency in mares. Eq. Vet. Sci. 4:301-308.

Woods, J., Bergfelt, D.R. and Ginther, O.J. 1990. Effects of time of insemination relative to ovulation on pregnancy rate and embryonic-loss rate in mares. Eq. Vet. J. 22(6):410-415.

Woods, J. and Ginther, O.J. 1983. Recent studies related to the collection of multiple embryos in mares. Theriogenology. 19:101 - 108.

XP-002103478, File Biosis, one page.

In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated by reference for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. However, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

Thus, the applicant(s) should be understood to have support to claim at least: I) the integrated herd management system described herein, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the application enhanced by the various systems or components disclosed, vii) the resulting products produced by such systems or components, ix) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, and x) the various combinations and permutations of each of the elements disclosed.

In addition, unless the context requires otherwise, it should be understood that the

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term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible in countries such as Australia and the like.

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VI. CLAIMS

I claim:

5 1. A method of managing animals, comprising the steps of:

- a. producing a female of a species of mammal;
- inseminating said female of said species of mammal with an artificial insemination sample having a plurality of spermatozoa, wherein at least 90% of said plurality of spermatozoa have a sex determination characteristic corresponding to the same sex of offspring mammal;
- fertilizing at least one egg within said female of said species of mammal;
 and
- d. producing said offspring mammal.
- 15 2. The method of managing animals as described in claim 1, wherein said sex determination characteristic comprises an X-chromosome.
 - 3. The method of managing animals as described in claim 1, wherein said sex determination characteristic comprises an Y-chromosome.

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- 4. The method of managing animals as described in claim 1, wherein said female of said species of mammal is selected from the group consisting of bovids, ovids, equids, canids, felids, and porcine.
- 25 5. The method of managing animals as described in claim 1, wherein said female of said species of mammal comprises a bovine species of mammal.
- 6. The method of managing animals as described in claim 5, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 10 million live non-frozen spermatozoa, no more than 5 million live non-frozen spermatozoa, no more than 3 million live non-frozen spermatozoa, no more than 1 million live non-frozen spermatozoa, no more than

500,000 live non-frozen spermatozoa, no more than 250,000 live non-frozen spermatozoa, and no more than 100,000 live non-frozen spermatozoa.

- 7. The method of managing animals as described in claim 5, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 10 million frozen-thawed spermatozoa, no more than 6 million frozen-thawed spermatozoa, no more than 5 million frozen-thawed spermatozoa, no more than 3 million frozen-thawed spermatozoa, no more than 1 million frozen-thawed spermatozoa, no more than 500,000 frozen-thawed spermatozoa, no more than 100,000 frozen-thawed spermatozoa.
 - 8. The method of managing animals as described in claim 1, wherein said female of said species of mammal comprises an equine species of mammal.
 - 9. The method of managing animals as described in claim 6, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 25 million live non-frozen spermatozoa, no more than 15 million live non-frozen spermatozoa, no more than 10 million live non-frozen spermatozoa, and no more than 5 million live non-frozen spermatozoa.

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- 10. The method of managing animals as described in claim 6, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 25 million frozen-thawed spermatozoa, no more than 15 million frozen-thawed spermatozoa, no more than 10 million frozen-thawed spermatozoa, and no more than 5 million frozen-thawed spermatozoa.
- 11. The method of managing animals as described in claim 1, wherein said plurality of spermatozoa comprises a number of spermatozoa from about 10% to about 50% relative to a typical number of spermatozoa in an artificial insemination sample.

12. The method of managing animals as described in claim 1, wherein said offspring mammal comprises a male, and wherein the percentage of male offspring mammals is selected from the group consisting of at least 70% male offspring mammals, at least 80% male offspring mammals, and at least 90% male offspring mammals.

The method of managing animals as described in claim 1, wherein said offspring mammal comprises a female, and wherein the percentage of female offspring mammals is selected from the group consisting of at least 70% female offspring mammals, at least 80% female offspring mammals, and at least 90% female offspring mammals.

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- 14. The method of managing animals as described in claim 1, further comprising the step of inducing early puberty in said female of said species of mammal.
- 15. The method of managing animals as described in claim 14, wherein said female of said species of mammal comprises a female of a bovine species of mammal, and wherein said step of inducing early puberty in said female of said bovine species of mammal comprises inducing puberty by nine months after birth.
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 16. The method of managing animals as described in claim 14, wherein said step of inducing early puberty in said female of said bovine species of mammal comprises inducing said early puberty between about 250 days after birth to about 270 days after birth.
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 17. The method of managing animals as described in claim 14, wherein said step of inducing early puberty in said female of said bovine species of mammal comprises feeding said female of said bovine species of mammal a sufficient ration of feed to produce an average weight gain of about 1.3 kilograms per day to about 1.4
 30 kilograms per day.
 - 18. The method of managing animals as described in claim 14, further comprising the

- step of early weaning of said female of said species of mammal.
- 19. The method of managing animals as described in claim 18, wherein said female of said species of mammal comprises said female of said bovine species of mammal, and wherein said step of early weaning comprises weaning said female of said bovine species of mammal at between about 95 days to about 125 days after birth.
- The method of managing animals as described in claim 18, wherein said step of inseminating said female of said bovine species of mammal comprises
 insemination of said female of said bovine species of mammal between about 283 days after birth to about 316 days after birth.
 - 21. The method of managing animals as described in claim 18, further comprising the step of synchronizing estrous.

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- 22. The method of managing animals as described in claim 21, wherein said step of synchronizing estrous comprises:
 - a. dressing feed with MGA at 0.5 milligrams per female of said bovine species of mammal per day for 14 days; and
- b. injecting PGF2 19 days after the last day of dressing said feed with said MGA.
 - 23. The method of managing animals as described in claim 22, further comprising the step of harvesting said female of said species of mammal.

- 24. The method of managing animals as described in claim 23, wherein said step of harvesting said female of said species of mammal comprises harvesting said female of said bovine species of mammal prior to 24 months.
- 30 25. The method of managing animals as described in claim 23, wherein said step of harvesting said female of said species of mammal comprises harvesting said female of said bovine species of mammal prior to 30 months.

26. The method of managing animals as described in claim 23, further comprising the step of replacing said female of said species of mammal with said offspring mammal.

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- 27. The method of managing animals as described in claim 3, further comprising the step of harvesting said male offspring mammal.
- 28. An animal management system, comprising:

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a. a number of females of a species of mammal, wherein each of said number of females of a species of mammal has no more than one parturition, and wherein said number of females are harvested prior to 24 months of age;

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b. a number of female offspring of said number of females of a species of mammal, wherein said number of female offspring are inseminated with a plurality of spermatozoa, and wherein a percentage of at least 70% of said plurality of spermatozoa have a sex determination characteristic corresponding to female offspring mammals, and wherein said number of female offspring replace said number of females of species of mammal harvested.

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29. The animal management system as described in claim 28, wherein said percentage of said plurality of spermatozoa have a sex determination characteristic corresponding to female offspring mammals is selected from the group consisting of at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, and at least 98%.

30. The animal management system as described in claim 28, wherein said female of said species of mammal is selected from the group consisting of bovids, ovids, equids, canids, felids, and porcine.

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31. The animal management system as described in claim 28, wherein said female of said species of mammal comprises a bovine species of mammal.

32. The animal management system as described in claim 31, wherein said wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 10 million live non-frozen spermatozoa, no more than 5 million live non-frozen spermatozoa, no more than 3 million live non-frozen spermatozoa, no more than 1 million live non-frozen spermatozoa, no more than 500,000 live non-frozen spermatozoa, no more than 250,000 live non-frozen spermatozoa, and no more than 100,000 live non-frozen spermatozoa.

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- The animal management system as described in claim 31, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 10 million frozen-thawed spermatozoa, no more than 6 million frozen-thawed spermatozoa, no more than 5 million frozen-thawed spermatozoa, no more than 3 million frozen-thawed spermatozoa, no more than 1 million frozen-thawed spermatozoa, no more than 500,000 frozen-thawed spermatozoa, no more than 250,000 frozen-thawed spermatozoa, and no more than 100,000 frozen-thawed spermatozoa.
- The animal management system as described in claim 28, wherein said female of said species of mammal comprises an equine species of mammal.
 - 35. The animal management system as described in claim 34, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 25 million live non-frozen spermatozoa, no more than 15 million live non-frozen spermatozoa, no more than 10 million live non-frozen spermatozoa, and no more than 5 million live non-frozen spermatozoa.
- 36. The animal management system as described in claim 35, wherein said plurality of spermatozoa comprises a number of spermatozoa selected from the group consisting of no more than 25 million frozen-thawed spermatozoa, no more than 15 million frozen-thawed spermatozoa, no more than 10 million frozen-thawed spermatozoa, and no more than 5 million frozen-thawed spermatozoa.

37. The animal management system as described in claim 28, wherein said plurality of spermatozoa comprises a number from about 10% to about 50 % relative to a typical number of spermatozoa in an artificial insemination sample.

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- 38. The animal management system as described in claim 28, further comprising an induced early puberty in said female offspring of said number of females of said species of mammal.
- 10 39. The animal management system as described in claim 38, wherein said number of females of said species of mammal comprise females of a bovine species of mammal and wherein said induced early puberty comprises said induced early puberty at a time no later than nine months after birth.
- 15 40. The animal management system as described in claim 39, wherein said induced early puberty in said female of said bovine species of mammal comprises said induced early puberty between about 250 days after birth to about 270 days after birth.
- 20 41. The animal management system as described in claim 40, herein said induced early puberty in said female of said bovine species of mammal comprises a sufficient ration of feed to produce an average weight gain of about 1.3 kilograms per day to about 1.4 kilograms per day per female of said bovine species of mammal.

- 42. The animal management system as described in claim 38, further comprising an early wean female of said species of mammal.
- The animal management system as described in claim 42, wherein said early wean female of said species of mammal comprises an early wean female of said bovine species of mammal, and wherein said early wean female of said bovine species of mammal is weaned at between about 95 days to about 125 days after birth.

44. The animal management system as described in claim 43, wherein said female of said bovine species of mammal are inseminated between about 283 days after birth to about 316 days after birth.

- 5 45. The animal management system as described in claim 42, further comprising synchronized estrous.
 - 46. The animal management system as described in claim 45, wherein synchronized estrous comprises:
- 10 a. top dressed feed with MGA at 0.5 milligrams per female of said bovine species of mammal per day for 14 days; and
 - PGF2 injection 19 days after the last day of top dressed feed with said
 MGA.
- 15 47. A method of managing animals, comprising the steps of:
 - a. producing a female of a species of mammal;
 - c. inducing early puberty in said female of said species of mammal;
 - c. inseminating said female of said species of mammal with a plurality of spermatozoa;
- 20 d. fertilizing at least one egg within said female of said species of mammal; and
 - e. producing a female offspring mammal.
 - 48. A method of managing animals, comprising the steps of:
- 25 a. producing a female of a species of mammal;

- b. weaning said female of said species of mammal early;
- inseminating said female of said species of mammal with a plurality of spermatozoa;
- d. fertilizing at least one egg within said female of said species of mammal; and
- e. producing a female offspring mammal.

- 49. A method of managing animals, comprising the steps of:
 - a. producing a female of a species of mammal;
 - inseminating said female of said species of mammal with a plurality of spermatozoa;
 - d. fertilizing at least one egg within said female of said species of mammal;
 - e. producing an offspring mammal; and

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- f. harvesting said female of said species of mammal prior to advance carcass maturity.
- 10 50. A method of managing animals, comprising the steps of:
 - a. producing a female of a species of mammal;
 - c. inducing early puberty in said female of said species of mammal;
 - inseminating said female of said species of mammal with a plurality of spermatozoa;
- d. fertilizing at least one egg within said female of said species of mammal; and
 - e. producing a female offspring mammal.
 - 51. A method of managing animals, comprising the steps of:
- 20 a. producing a female of a species of mammal;
 - b. weaning said female of said species of mammal early;
 - inseminating said female of said species of mammal with a plurality of spermatozoa;
 - d. fertilizing at least one egg within said female of said species of mammal;
- e. producing a female offspring mammal.
 - 52. A method of managing animals, comprising the steps of:
 - a. producing a female of a species of mammal;
 - inseminating said female of said species of mammal with a plurality of spermatozoa;
 - d. fertilizing at least one egg within said female of said species of mammal;
 - e. producing an offspring mammal;

f. harvesting said female of said species of mammal prior to advance carcass maturity.

- 53. A method of managing animals, comprising the steps of:
- 5 a. inseminating a female mammal;
 - inseminating said female of said species of mammal with a plurality of spermatozoa, wherein at least 90% of said plurality of spermatozoa bear a Y-chromosome;
 - b. fertilizing at least one egg within said female of said species of said mammal; and
 - c. producing a male offspring mammal.

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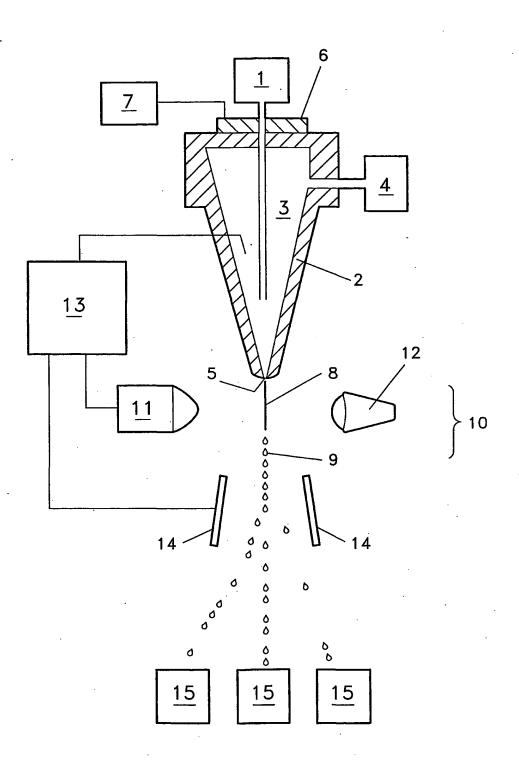


Fig. 1 SUBSTITUTE SHEET (RULE 26)

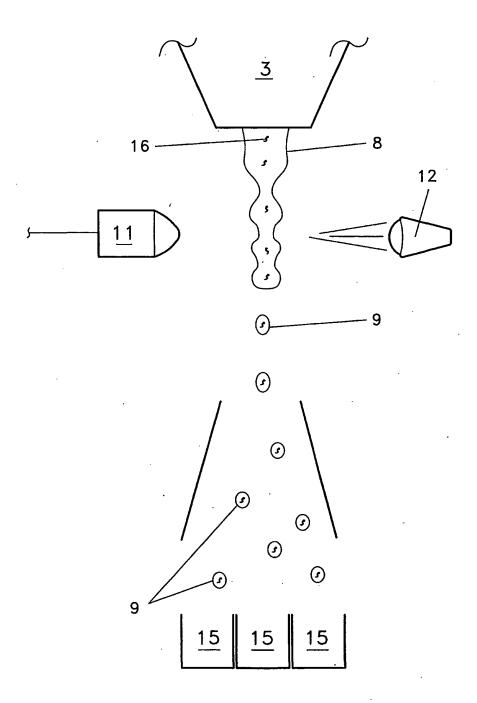
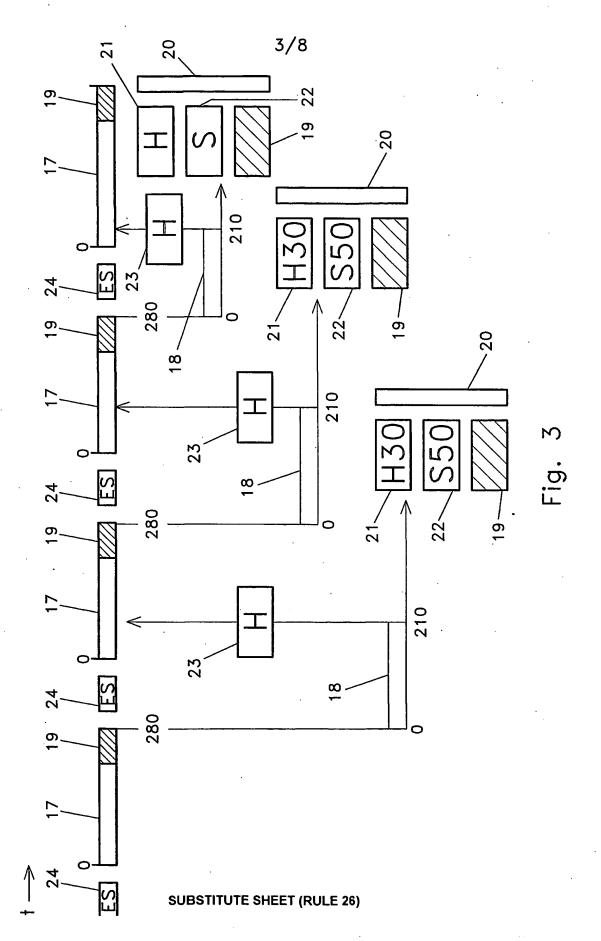
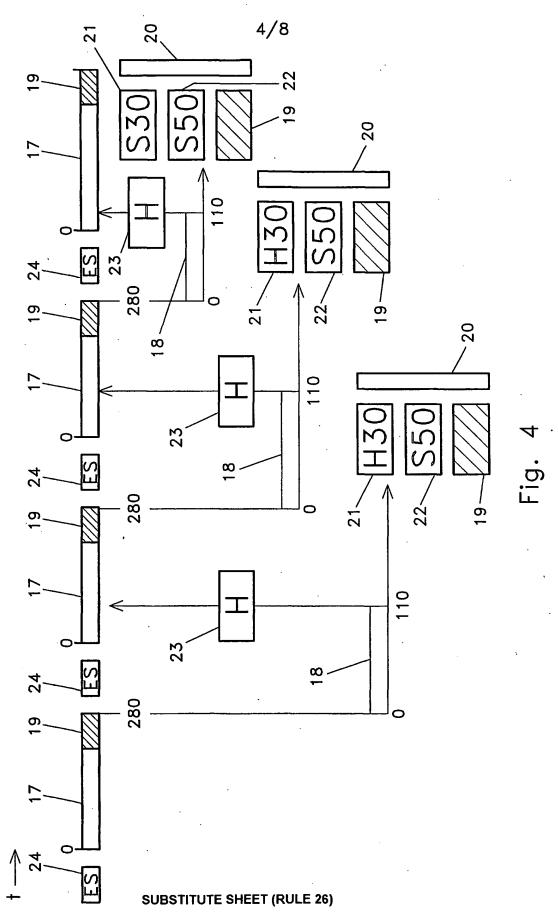
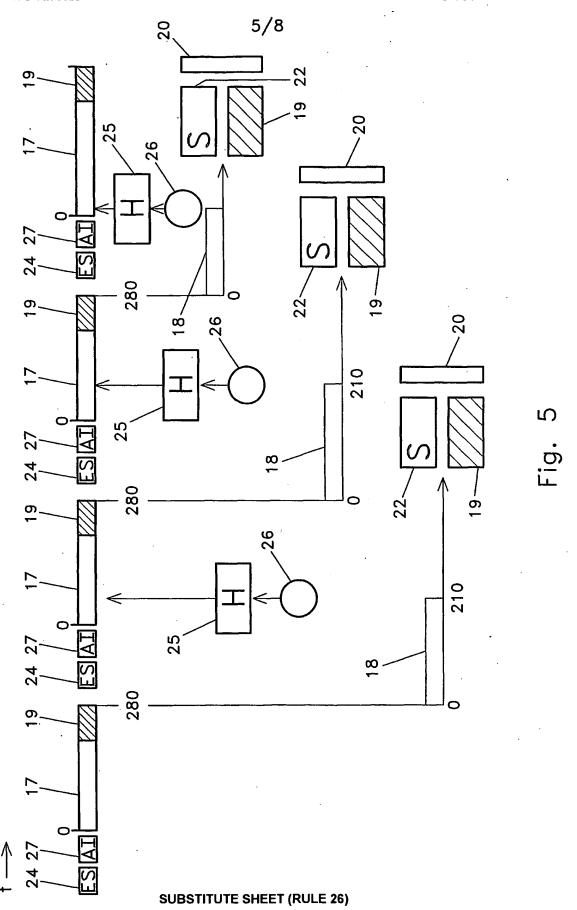


Fig. 2 SUBSTITUTE SHEET (RULE 26)

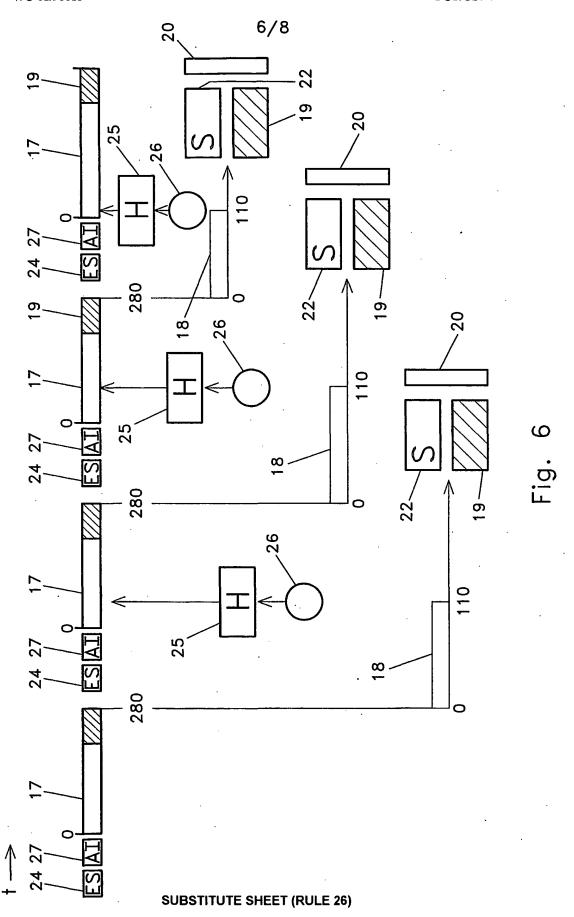


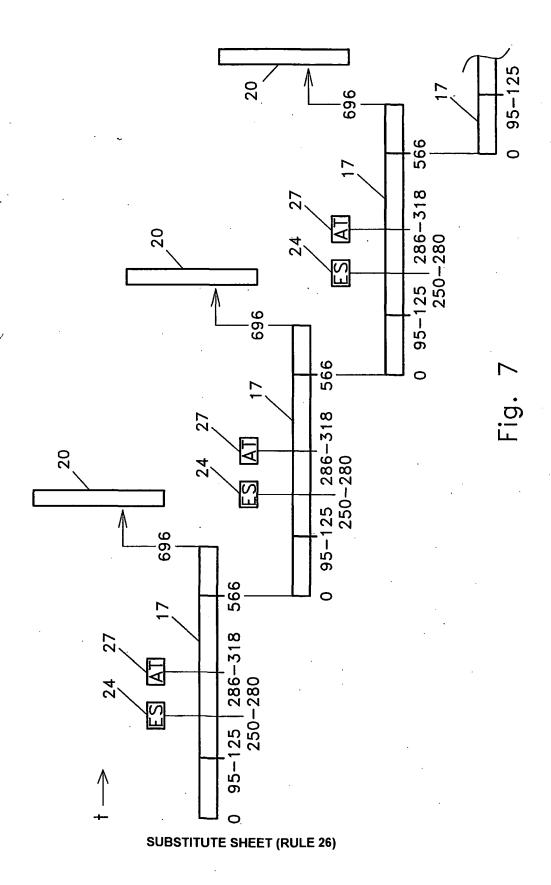
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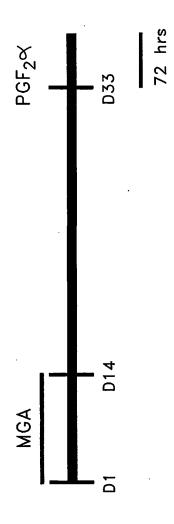




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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/18879

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61B 17/43; A61D 7/00 US CL : 600/35 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S.: 600/35, 34, 33; 128/898; 514/178; 435/2, 7, 7.21; 424/105, 78, 85, 561, 185.1				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WEST: artificial insemination, bovine, equine, induced puberty, sex characteristic, sperm separation, flow cytometer				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category *	Citation of document, with indication, where ap	opropriate, of the relevant passages	Relevant to claim No.	
X.	US 4,327,177 A (SHRIMPTON) 27 April 1982, see whole document		1-13, 52, 53	
Y Y			47, 48, 50, 51	
Y,P	US 6,087,352 A (TROUT) 11 July 2000, see whole document.		14-17, 47, 48, 50, 51	
Further	documents are listed in the continuation of Box C.	See patent family annex.		
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